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A Supplement to the Demarcation Lines of Pigmentation Observed among the Japanese, on Inner Sides of Their Extremities and on Anterior and Posterior Sides of Their Medial Regions

By

Osamu Miura
(三浦 修)

(From the Department of Dermotology, Faculty of Medicine, Nihon Univ., Tokyo. Director: Prof. O. Miura)

(Reeceived for publication, Nobember 7, 1951)

In the communication from Dr. Akio Maruyama on the demarcation lines of pigmentation under the above subject he writes about his further experience on this pigment anomaly. At this opportunity I tender my heartfelt thanks to him for his friendship.

His observation was as follows: In a 21 years old young man who came for health examination at the ambulance a demarcation line of pigmentation, besides the one which belongs to the type III of the upper arm groups classified by me in my previous report,[1] was observed on the posterior side of his upper arms as in Fig. 1. In this case the former was localized in only the chest, i.e. it was an incomplete form (Fig. 2). The details of the latter based on the sketches and on the brief description by Dr. A. Maruyama are: the demarcation line begins from the middle part of the upper arm, ascends the arm almost between the long head and the lateral head of the triceps, then arches at the upper border of the axilla and stops there. The two ends of the line fade naturally into a normal skin color. As for the difference in the color tone, when the arm is held up in a horizontal position the part under the line is pale.

Previously, I reported in this Journal[1] an account of the demarcation lines described above and offered at that time that the demarcation lines of pigmentation coincide exactly with the parts where there is no duplication of Sherrington's dermatoms, i.e. that the lines coincide exactly with the axial line of the extremities and with the medial region of the trunk. However, in summarizing all the reports published sofar there is no description of the demarcation line which is on the posterior side of the extremities, i.e. the one coinciding with the posterior axial line. This was the weak point of my popinion.

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Fig. 1

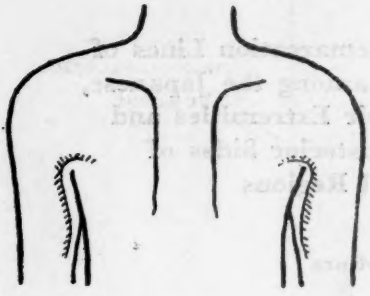
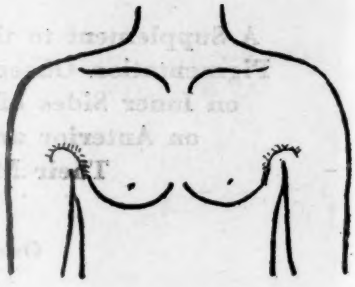


Fig. 2



Now I can add a new fact. It is that it was proved that the demarcation line corresponding to Sherrington's axial line on the posterior side of the upper extremities actually exists. However, to my regret, the demarcation line of the lower extremity corresponding to that of the arm mentioned above has not been observed yet.

On this occasion I wish add the following: Both A. Maruyama[2] and K. Maruyama[3] pointed out in their reports that the demarcation lines were observed more frequently in patients with tuberculous diseases than in patients with other diseases. A. Maruyama proved statistically that this phenomenon is seen in 15% of the patients suffering from pleurisy or tuberculous lung disorders, but with the diseases of other nature this parcentage is lower, while in the healthy the rate is only 3%. So they supposed that this phenomenon might be one of the characteristics of tuberculous diseases. Recently, Harada[4] reported also a similar result. Regarding this hypothesis I myself have nothing to write here now.

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Effect of Glutamic Acid, NaCN and Monoiodoacetic Acid on the Potential Changes of Isolated Toad's Brain

By

Mituru Ebe
(江 部 充)

(From the Physiological Laboratory of Prof. K. Motokawa, Tohoku University, Sendai)

(Received for publication, November 15, 1951)

Hayashi[1] reported that a convulsive seizure can be induced on dogs, by injecting a very minute amount of sodium salt of glutamic acid into the cortex of the brain. We also observed a similar phenomenon caused by injecting the drug into the brain or the spinal cord of a frog or a toad. The present experiment was undertaken in order to investigate the effect of glutamic acid upon the potentials of the isolated toad's brain. The effects of NaCN and monoiodoacetic acid were also studied in comparison with that of glutamic acid.

Experimental

Method

The toad was decapitated without anesthesia. The dorsal skin was reflected, the skull twisted with a pair of a sturdy scissors and the brain exposed. The cranial nerves and spinal cord were cut off from the brain, which was, then, gently taken out. In this procedure it was carefully avoided to injure or to distort the brain. The brain so isolated was, after having been washed in Ringer's solution, placed on a piece of filter paper moistened with Ringer's solution in a small dish. Cotton thread electrodes were used for leading off potential changes, one of them being placed on the surface of the brain and the other on the filter paper. The whole procedure had to be carried out as rapidly as possible; we used to finish the procedure within a few minutes.

Glutamic acid was solved in water. NaCN and monoiodoacetic acid were solved in Ringer's solution, and the H-ion concentration was corrected to neutral with phosphate buffer. All experiments were carried out at room temperature of 18 to 20°C.

Results

Spontaneous Activity. Libet and Gerard[2] found that at high temperature the electrical activity of an isolated frog's brain was large in amplitude, but rapidly disappeared, while at low temperature the amplitude was very small. In our experiment, at moderate room temperature the spontaneous waves lasted for ten minutes or longer. Though the rhythm was found variable from case to case, the frequency was in general 4 or 5 cycles per second, and the amplitude 10 to 50 μ V. The change in amplitude after isolation of the brain is graphically shown in Fig. 1, a. Immediately after isolation the amplitude was largest and gradually de-

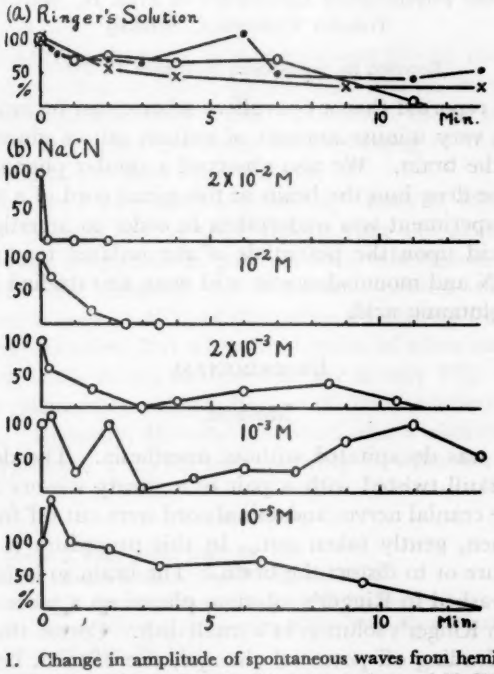

Fig. 1. Change in amplitude of spontaneous waves from hemisphere of isolated toad's brain after soaking in Ringer's solution and NaCN-Ringer's solution: (a) three cases in Ringer's solution, pH 7.2. (b) in NaCN-Ringer's solution, pH 7.0.

creased with the lapse of time (Fig. 3.A). The spontaneous waves thus reduced in amplitude were meanwhile replaced by diphasic spike potentials, which eventually faded into the noise level. The amplitude was found large on the olfactory bulb, hemisphere, and optic lobe in order.

Glutamic Acid. As soon as the brain was soaked in 1 per cent glutamic acid solution, so-called convulsive waves appeared on the hemisphere and the optic lobe, and lasted for several seconds or longer; they were over 100 μ V in amplitude and 10 to 20 cycles per second in frequency (Fig. 2).

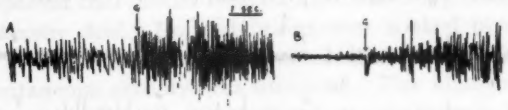

Fig. 2. Convulsive waves evoked by 1 per cent glutamic acid solution from hemisphere of isolated toad's brain. (A) in the midst of spontaneous discharges. (B) after fading of spontaneous discharges.

Then, the convulsive waves were replaced by potentials of irregular rhythm which were finally followed by spike potentials. The minimal concentration just necessary to evoke convulsive waves was different from region to region of the brain. For example, on the hemisphere a drop of 0.1 per cent solution from an injector of 1/4 mm. in diameter was sufficient to evoke convulsive waves, and on the optic lobe a drop of 1 per cent was the threshold. The olfactory bulb was, however, found so insensitive to this drug that no appreciable convulsive waves could be obtained from it. Convulsive waves could be elicited by the drug even after the spontaneous discharge had disappeared. This indicates that glutamic acid is a strongly stimulating agent of the brain. Repeated application of a drop of the solution to the hemisphere at intervals of 10 minutes caused a burst of convulsive waves every time, and such responses could be found till about 2 or 3 hours after excision of the brain. Such a procedure may be used for testing the excitability of the brain. Convulsive waves were also evoked in such a manner that a piece of filter paper of 1 mm. square moistened with the drug was placed on the brain. The waves evoked in this manner were transmitted from one part to another.

H-ion Concentration. The brains were soaked in Ringer's solution, of which the pH values were varied with phosphate and HCl buffer from 11.0 to 3.0. The amplitude of spontaneous waves increased in alkaline solution and decreased in acid solution within the pH range from 8.0 to 3.0. Soaking in an alkaline solution of pH 11.0 set up strong convulsive waves similar to those evoked by glutamic acid. In an acid solution of pH 3.2 spontaneous waves were strongly reduced in amplitude, but the excitability of the brain as tested by application of glutamic acid was not so strongly affected, as can been seen from the following experiments: the response of the brain to stimulation with a drop of 1 per cent glutamic acid could be found even after soaking in this solution for about 3 hours.

NaCN. We investigated quantitatively the relation between the amplitude of spontaneous waves and the concentration of NaCN. The concentrations of NaCN-Ringer's solutions were varied from 2×10^{-2}M to

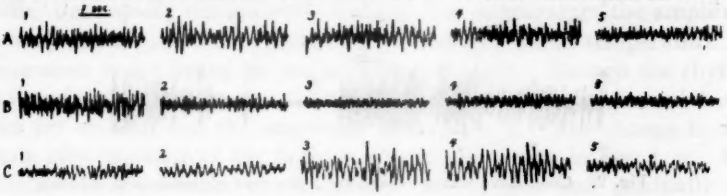

Fig. 3. Change of spontaneous waves from hemisphere of isolated toad's brain after soaking in various solution. (A) in Ringer's solution: 1. 3 min. after isolation. 2. 7.5 min. 3. 9 min. 4. 13 min. 5. 21 min. (B) in NaCN-Ringer's solution of 2×10^{-3} M.: 1. normal control in Ringer's solution 4 min. after isolation. 2. 1.5 min. after soaking in NaCN. 3. 3 min. 4. 4 min. 5. 10.5 min. (C) in monoiodoacetic acid-Ringer's solution of 10^{-4} M. 1. normal control in Ringer's solution 4 min. after isolation. 2. 1.5 min. after soaking in monoiodoacetic acid. 3. 2.5 min. 4. 4.5 min. 5. 8.5 min.

10^{-5}M. The brains were soaked in these solutions. As shown in Fig. 1, b, the spontaneous waves of the hemisphere were immediately abolished in 2×10^{-2}M solution, and in 10^{-2}M, however, 3 minutes after soaking. In 2×10^{-3}M and 10^{-3}M solutions the spontaneous waves were reduced in amplitude or abolished only temporarily and recovered afterwards to the same level as in control (Fig. 3. B). In a solution of much lower concentrations such as 10^{-5}M, spontaneous waves, after an initial rise, took a time course similar to normal control soaked in Ringer's solution.

Gerard and Libet[3] reported that NaCN was generally less effective for reducing spontaneous waves in frogs than in other animals and that the drug acts occasionally as a stimulating agent. In our experiments spontaneous waves were irreversibly reduced in higher concentrations of NaCN and abolished temporarily in intermediate concentrations, but in lower concentrations this drug brought about an initial rise of amplitude, which is presumably due to a stimulating action of NaCN. It is likely that the brain of frog is less sensitive to NaCN than those of higher animals, and as a consequence the drug must act stimulating on the frog's brain even at such a high consentration that a paralysing action would be seen on the brains of other animals. In order to study more quantitatively the excitability of the frog brain soaked in NaCN solution, the following experiment was performed. One drop of 1 per cent glutamic acid solution at a time was repeatedly applied to the hemisphere at intervals of 10 minutes till the brain ceased to respond to the stimulation. It was found that the

brain soaked in 2×10^{-2}M solution gave no response to glutamic acid and that the brain soaked in 10^{-2}M solution could respond only to the first stimulation. At 2×10^{-3}M responses could be seen till 20 minutes after soaking, and at 10^{-3}M till 50 minutes. Thus, the lower the concentration of NaCN, the longer remained the brain excitable. From these observations it is apparent that NaCN reduces the excitability of the brain.

Monoiodoacetic Acid. Isolated brains were soaked in monoiodoacetic acid-Ringer's solutions of various concentrations from 10^{-2}M to 10^{-5}M, and the spontaneous activity was observed. The changes in amplitude of the spontaneous waves from the hemisphere are shown in Fig. 4. Mono-

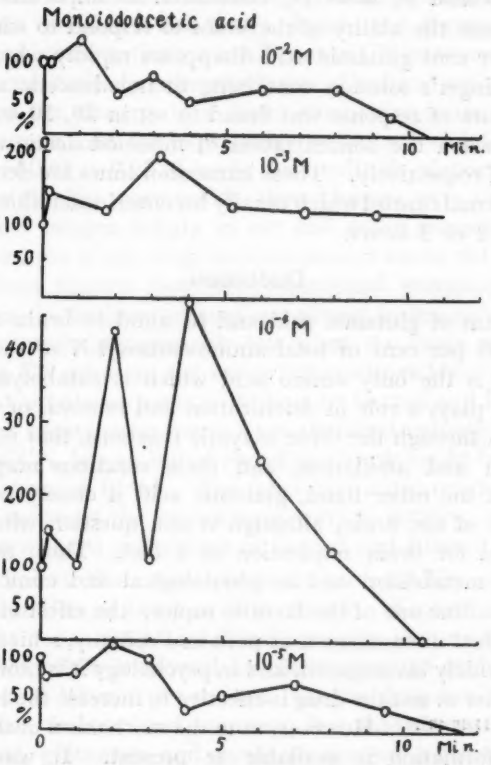

Fig. 4. Change in amplitude of spontaneous waves from hemisphere of isolated toad's brain after soaking in monoiodoacetic acid-Ringer's solution, pH 7.0.

iodoacetic acid of 10^{-2}M had no effect on the amplitude. In 10^{-3}M and 10^{-4}M solutions the amplitude increased conspicuously and the rhythm

became more regular (Fig. 3. C). The solution of 10^{-5}M seemed to have no effect on the spontaneous waves, which continued as long as in normal control.

Gerard and Libet[8] described the appearance of polyphasic irregular waves from frog's brain soaked in iodoacetate, but in our experiment the rhythm was found always regular at least in the concentration range tested so far. Thus, the drug either has no effect upon the spontaneous waves or enhances them in moderate concentrations. It seems, therefore, that spontaneous waves have little to do with carbohydrate metabolism which is supposed to be impaired in the presence of monoiodoacetic acid. Carbohydrate metabolism is, however, considered as important for recovery processes, because the ability of the brain to respond to stimulation with a drop of 1 per cent glutamic acid disappears rapidly when the brain is soaked into Ringer's solution containing monoiodoacetic acid. For example the failure of response was found to set in 20, 30 and 70 minutes after soaking when the concentration of monoiodoacetic acid was 10^{-2}, 10^{-3} and 10^{-4}M respectively. These immersion times are decidedly shorter than that of normal control which usually becomes inexcitable with glutamic acid in about 2 or 3 hours.

Discussion

The amount of glutamic acid and its amid in brain are said to be about 40 to 80 per cent of total amino-carboxyl-N of the brain,[4] and glutamic acid is the only amino acid which is catabolyzed in brain.[5] Glutamic acid plays a role in deionization and removal of ammonia produced in brain through the three enzymic reactions, that is, deamination, transamination and amidation, and these reactions may rapidly proceed.[6][7][8] On the other hand, glutamic acid is closely concerned with the respiration of the brain, although it is a question whether glutamic acid is utilized for brain respiration as a fuel. Thus, the problem of glutamic acid metabolism and its physiological and clinical significance has recently become one of the favorite topics; the effect of glutamic acid upon neurological diseases, such as petit mal epilepsy, schizophrenia, mongolism etc. is widely investigated, and in psychology it is now an interesting question whether or not the drug is effective to increase the learning ability of animals.[9][10][11][12][13][14] However, as to the mechanism of the effect of the drug, little information is available at present. It was reported by Nachmansohn[15] that synthesis of acetylcholine (Ach) in dialyzed brain extract increased obviously in the presence of glutamic acid. Krebs and Eggleston[16] showed that glutamic acid had an effect on the permeability of the neuronal membrane, especially permeability to K ions. Nachmansohn[17] suggests that Ach plays an important role in producing action

potentials of nervous tissues, by changing the permeability of the neuronal membrane to K ions. Consequently it may be possible that glutamic acid increases the activity of Ach by facilitating its synthesis. In our experiment the potentials which were evoked by Ach in the isolated toad's brain were found to be very similar to those evoked by glutamic acid; strong convulsive waves are caused by a drop of Ach in concentrations of 10^{-1} to 10^{-4} per cent.

Let us now discuss the effect of NaCN. It is well known that the cytochrome-cytochrome oxydase system in brain is inhibited by CN.[18)19)20)] According to Marshall and Rosenfeld,[21)] respiration of animals is enhanced by injection of CN in a small dose, probably because of the high sensitivity of the sino-aortic receptor to this drug. Rubin and Freeman[22)] observed that in 19 schizophrenic patients a small dose of CN intravenously injected increased the amplitude and the regularity of cortical activity and these authors ascribed the changes to a direct stimulating action of CN upon the cortical neurons. In the experiment by Gerard and Libet,[3)] CN in concentrations of 0.03 to 0.05 M made the amplitude of spontaneous waves smaller and the rhythm more rapid; these changes were similar to those due to decreased oxygen supply in cat and other animals. Moreover, in high concentration of this drug the spontaneous waves did not disappear completely. Even though they were suppressed temporarily, they recovered. When the brain so treated was placed in Ringer's solution, the brain waves promptly disappeared, but when the brain was again immersed into the CN solution, the waves returned quickly. However, our observations did not always agree with those by Gerard and Libet. In our experiments, the spontaneous waves were abolished entirely in concentration of 10^{-2}M of CN, but an initial rise of amplitude could be seen in such a low concentration as 10^{-5}M. The latter fact indicates that CN in low concentrations enhances the spontaneous cortical activity, as is suggested by the above mentioned experiment of Gerard and Libet.

It is well known that monoiodoacetic acid inhibits glycolysis and disturbs the formation of lactic acid in muscle. Krebs[23)] informed that iodoacetic acid of 5×10^{-4} M inhibited strongly the oxidation of glucose in the brain, while it showed no effect upon the oxidation of lactic acid. Quastel and Wheatley[24)] showed that the drug inhibited the oxidation of glucose, fructose and mannose, but that the oxidation of dl-lactate, pyruvate, glutamate and succinate was not affected by it. According to Gerard and Libet,[3)] when the isolated frog's brain was soaked in an iodoacetate solution, regular waves of diminished amplitude were seen which were from time to time replaced by discharges of large amplitude and irregular profil. In our experiments, however, monoiodoacetic acid increased strongly the amplitude and regularity of spontaneous

waves, but no such occasional bursts of irregular large waves as described by Gerard and Libet could be seen. This increasing action of monoiodoacetic acid upon the cortical activity is far more conspicuous than that of CN described above, and a depressant effect could hardly be seen with concentrations so far tested. However, its injurious effect could clearly be seen in that the brain treated with monoiodoacetic acid loses the capacity to respond to glutamic acid with the characteristic convulsive waves far sooner than the normal control.

Summary

1. Spontaneous potential waves from isolated toad's brain had the amplitude of 10 to 50μ V and the frequency of 4 or 5 cycles per second.

2. When glutamic acid was applied to the brain, convulsive waves over 100μ V in amplitude and of 10 to 20 cycles per second were evoked from the hemisphere and the optic lobe. With regard to the threshold concentration of glutamic acid, some local differences were found.

3. The amplitude of spontaneous waves increased in alkaline and decreased in acid Ringer's solutions. At extremely high values of pH such as 11.0 there occurred convulsive waves similar to those evoked by glutamic acid.

4. NaCN abolished the spontaneous waves in high concentrations, but enhanced them temporarily in low concentrations.

5. Monoiodoacetic acid increased the amplitude of the spontaneous waves in middle concentrations, while no change was seen in higher or lower concentrations.

6. Repeated application of a drop of 1 per cent glutamic acid to the hemisphere at intervals of 10 minutes caused a burst of convulsive waves every time, and such a response could be found till about 2 or 3 hours after excision of the brain. The brain soaked in NaCN, however, soon lost the ability of responding to stimulation with glutamic acid. Monoiodoacetic acid showed a similar effect even in such moderate concentration in which the drug enhanced the spontaneous waves.

Finally I express here my hearty thanks to Prof. Motokawa who furnished me with guidance and helpful criticism.

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"Hepatogenous Convulsions" and "Hepatic Anticonvulsant"

Preliminary Report

By

Akira Sato

(佐藤　彰)

(From the Department of Pediatrics, Tohoku University, Sendai)

(Received for publication, June 14, 1952)

Epileptic children are made free of seizures (Cf. Takamatsu and Sato[1] 1940) by yakriton, at least for some months. Dr. Amamiya published the same result quite independently of, and 2 years prior to, those authors. What is the reason for its anti-epileptic effect?

Yakriton is the detoxicating hormone of the liver and can detoxicate not only ammonia, but also many other poisons. Amamiya ascribed its anti-epileptic effect to the detoxication of cardiazol (or epileptogenic poison). Takamatsu and Sato took also other possibilities into consideration. Recently I have begun to think of another possibility, which will offer the most plausible explanation for the anti-epileptic effect.

I was able to succeed in isolating yakriton[2] (1926) from the liver, only after I had found that there were at least two kinds of normal rabbits according to the ammonia detoxicating ability. A rabbit of *b*-classed liver power will develop general convulsions (apparently almost fatal) within 15 minutes of 3% ammonium chloride, while another of *f*-classed or high-classed liver power will remain quite healthy in spite of the injection. This is *ammonia convulsions* indeed, but from the viewpoint of liver, it is "hepatogenous or hepatogenic convulsions."

To produce the "anencephalic blood picture" or "the contra-striatal blood picture" (Sato and Kuribayashi)[3] in rabbits, hemispheres were removed. At this attempt Kuribayashi made experiements on both—*b*- and *f*-classed—kinds of animals. This differentiation had been thought unnecessary at this experiment, because the blood picture ensuing would have nothing to do with the ammonia detoxicating liver power.

The result was astonishing. The blood picture was a monstrous one; it was however produced equally in *b*- as well as *f*-classed rabbits. But the two kinds of animal died after a survival of several days in a different manner. *f*-classed rabbits died calmly, whereas *b*-classed animals *died with general convulsions*.

I desire to suggest "hepatogenous or hepatogenic convulsions" for such convulsions. And epileptic children were made free of seizures by yakriton —"hepatic anticonvulsant", while their liver had been made "stronger" The details will be reported in the near future.

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The Existence of Co-carboxylase in Human Milk*

Part 1. Infuence of Human Milk upon Decarboxylation of Pyruvate by Alkaline Washed Yeast Carboxylase

218th Report of the Peroxidase Reaction

(165th Human Milk Study)

By

Tsuneo Arakawa

(荒川 雅男)

(From the Department of Pediatrics, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. A. Sato)

(Received for publication, November 16, 1951)

In 1938 Ochoa and Peters[1] reported a method for the determination of carboxylase and free vitamin B_1 in animal tissues by means of decarboxylation of pyruvate by alkaline washed yeast. Tauber,[2] however, stated that there was no direct proportionality between cocarboxylase concentration and CO_2 formation from pyruvate, and further that the carboxylase content of various yeasts differed greatly. For these reasons he wrote that it was difficult to make an exact comparison of the cocarboxylate activity by the enzymatic test according to Ochoa and Peters,[1] method.

Recently, in March 1940, Greenberg and Rinehart[3] stated that sulfhydryl and other reducing compounds had a stimulating effect on the decarboxylation activity of cocarboxylase and that therefore the assay of tissues and biological fluids for cocarboxylase by the method of Ochoa and Peters[2] might be open to question, since most tissues were rich in SH-compound.

EXPERIMENTAL

My method used was a modification of Ochoa and Peters,[1] method. As a source of carboxylase was used commercial baker's yeast (Fleischmann) which had been air-dried with the aid of an electric fan for 5 hours at 30°C. One gram of this dried yeast was freed from cocarboxylase by rapid washing according to Ochoa and Peters'[1] description, and sus-

* Arakawa began to suspect the existence of cocarboxylase in human milk in summer 1937, but with the thither to listerature stating to the contrary, he postponed its publication at that time. He worked further on the problem in collaboration with Naito, but had to leave the Laboratory July 1941. The present work was completed in 1938, (Cf. A. Sato, the Tohoku Igaku Zasshi, 1942, **31**, 409).

pended in 10 cc. of phosphate buffer of pH 6.2. Formation of CO_2 from pyruvate was measured manometrically at 28°C in air with Barcrofft's differential manometer. Each vessel contained 1.0 c.c. of washed yeast suppension, 0.1 mgr. Mg as $MgCl_2$ in 0.1 c.c. of solution. 0.2 c.c. of sodium pyruvate solution adjusted to pH 6.2 and containing 5 mgrms. of pyruvic acid, 10γ of oryzanin in 0.1 c.c. of solution, and 0.5 c.c. of human milk to be tested.

Results

TABLE

Difference of Human Milk Samples of Different Arakawa's Reaction on Decarboxylation of Pyruvate by Alkaline Washed Yeasts

Date of experiment	No. of experiment	Arakawa's reaction 0′	1′	2′	3′	4′	5′	Microliters CO_2 liberated in 30 minutes
22. VII	1	±	++	+++	+++	+++	+++	13
	2	+	+++	+++	+++	+++	+++	22
	3	−	−	−	−	−	−	10
	4	−	−	−	−	−	−	0
23. VII	5	−	+	+	+	++	++	25
	6	−	++	++	+++	+++	+++	32
	7	−	−	−	−	−	−	37
	8	−	−	−	−	−	−	16
	9	−	+	+	+	+	++	22
	10	+	++	+++	+++	+++	+++	41
	11	−	++	+++	+++	+++	+++	67
25. VII	12	+	++	+++	+++	+++	+++	26
	13	−	±	+	++	++	++	38
	14	±	++	++	+++	+++	+++	41
27. VII	15	±	+	++	++	++	++	32
	16	−	−	−	−	±	+	15
	17	−	+	++	++	+++	+++	40
	18	−	+	++	++	++	+++	50

On judging the results given in the above table, the volumes of CO_2 liberated from pyruvate must be compared with each other only on the same experimental day, because there will be great difference in the content of carboxylase of alkaline washed yeast prepeared on each experimental day, as mentioned in the Hauber's[2] report.

As will be seen from by investigation (Cf. Table), it is generally concluded that milk with positive Arakawa's reaction will have more stimulating effect on CO_2 formation from pyruvate by alkaline washed yeast than the milk negative to Arakawa's reaction and further this relation was observed between on side milk that from the other side milk of the same individual.

SUMMARY AND CONCLUSIONS

1) I examined the effect of human milk with different Arakawa's reaction on the decarboxylation of pyruvate by alkaline washed yeast according to Ochoa and Peter's method.

2) In human milk tested there was found no substance inhibiting decarboxylation of pyruvate by alkaline washed yeast.

3) The milk positive to Arakawa's reaction showed more stimulating effect on the decarboxylation of pyruvate by alkaline washed yeast than the Arakawa negative milk.

4) Whether the stimulating effect of human milk on CO_2 formation from pyruvate is to the cocarboxylase itself or other stimulating substance which will be contained in human milk, is not yet settled, though the existence of cocarboxylase in human milk is probable.

This work was carried out by a Grant from the Foundation for Promotion of Scientific Research of the Ministry of Education. Prof. Akira Sato.

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Further Reports on the Relation between Arakawa's Reaction and Thiamine Content of Human Milk

Part 1. Free Thiamine of Human Milk*

116th Report of the Peroxidase Reaction

(173rd Human Milk Study)

By

Takeshi Natio

(内 藤 武)

(From the Department of Pediatrics, Faculty of Medicine, Tohoku University, Sendai, Director: Prof. A. Sato)

(Received for publication, November 16, 1951)

Since Jansen applied the thiochrome method to thiamine determination, a number of papers concerning the original itself and its modifications have appeared. As to the thiamine of human milk only a few papers (up to this writing) have been published. Neuweiler[1] states that thiamine content of human milk in the 2nd–20th week after delivery lies between 0–13 γ mostly somewhere near 10 γ. Hirai and Ide[2] reported that thiamine content of human milk was about 5–10 γ. According to Widenbauer and Heckler[3] it is 2–36 γ and on an average 10 γ. Ts. Arakawa[4-5] in our Laboratory investigated for the first time the relation between Arakawa's reaction and thiamine content of human milk. He took a great deal of trouble to exclude non-specific substances giving fluorescence and found the thiamine content of human milk to be 0–15 γ (17.5 γ on an average). In this study of his he came to the very important conclusion that human milk with positive Arakawa's reaction was of higher thiamine content than Arakawa-negative milk.

Now I desire to report my own results which I have obtained by using my own modification of Arakawa's[4] method above mentioned. In Part 1 of the present treatise I shall report of the amount of free thiamine in human milk.

Materials

My milk samples were mostly obtained from mothers of those breast-fed

* Read at the General Meeting of the Japanese Pediatric Society, Tokyo, 28–30 March, 1942; in Part 2 of this treatise the phosphate bound thiamine of human milk will be reported, This form of thiamine was shown for the first time by Ts. Arakawa (he left our Laboratory July, 1941, shortly after he had worked on this thema).

infants who visited our Pediatric Department or were admitted to it. Such lactants, however, who had been taking some thiamine preparation, were excluded. Also such mothers with a new pregnancy or with menstruation or mastitis and lactants within 3 weeks of their delivery were excluded.

Method of Experiment

1. *Arakawa's reaction*

Milk samples were tested with the reaction within 3 hours of obtaining and observed during 5 minutes.

2. *Determination of free thiamine*

Milk samples were examined as early as possible, within 3 hours at the latest. As to the method of determination Arakawa's and Wang's modifications were used with a slight modification of my own. These give, I believe, fairly precise results, as far as human milk is concerned.

Method: Centrifuge a milk sample (2500 rotations per minute for 30 minutes) and take 1.0 cc. of the skimmed human milk. Add (in most cases) 3 drops of N/4 HCl solution until pH 3.5 is reached. Keep it in water bath of 65°C for 8 minutes, add 0.1 grm. of pepsin (Merck), and keep it at 37°C in incubator for 12–16 hours. Add ca. 8 cc. of HCl solution of pH 3.5, 10 cc. of isobutanol and 10 cc. of ether. Shake it vigorously for ca. 5 minutes and pour it into separating funnel. Soon the underlayer will become quite water-clear. After one hour pour the clear under-layer into 10 cc. volumetric flask and add HCl solution of pH 3.5 up to the mark. One part of it will be used for the preparation of the test solution (a) and the other for the control (b).

a) Preparation of the test solution

Take 5.0 cc. (—any remaining part—even drops—should be put back into the volumetric flask) of the solution above described, add 10 cc. of HCl solution of pH 3.5, and the 0.5 cc. of 1% potassium ferricyanide solution, immediately followed by addition of 15% NaHO solution. Mix only gently for 2 minutes. Add 15.0 cc. of isobutanol and then shake vigorously for 2 minutes. After separation pipette the isobutanol layer into a separating funnel (see below).

b) Preparation of the control solution

The other part of the solution remaining in the volumetric flasks (see above), 5 cc. in amount, is poured into a flask with rinsings made with 10 cc. of HCl solution of pH 3.5. Add 10 cc. of 15% NaHO and 15 cc. of isobutanol and shake vigorously for 2 minutes. Then pour the isobutanol layer into a separating funnel (see below).

Add to both preparations—the test solution as well as the control—each in a separating funnel 10 cc. of water and shake vigorously for one minute in order to wash the butanol and dry this with sodium sulfate.

Filter.

c) Preparation of standard thiochrome solution

Put 2.0 cc. of 500 γ/dl solution of oryzanin (cyrstals of thiamine hydrochloride, Sankyo) in

a glass stoppered flask of 25 cc. To this add 0.1 cc. of 0.1% potassium ferricyanide solution (Merck) and 2 cc. of 15% NaHO solution. Mix the content for 2 minutes gently, then add 10 cc. of isobutanol and mix for 2 minutes. Separate the isobutanol layer and dry by means of shaking with sodium sulfate anhydrate. Then filter. 1.0 cc. of this standard thiochrome thus obtained solution will show the intensity of fluorescence corresponding to 1 γ of thiamine hydrochloride. The preparation is thus the same as described in Arakawa's paper).[4]

Put each 6.0 cc. of the test resp. crystal solution into a glass-stoppered tube with a diameter of 1 cm. and compare the intensity of fluorescence both solutions according to Ritsert's description under the ultra violet light. Each of 0.005 cc. of the standard solution (corresponding to 2.5 γ/dl thiamine hydrochloride content) is added at one time to the control.

TABLES I

Free Thiamine Content of Human Milk with the Better Intermediate Arakawa's Reaction

No. of specimen	Age		Arakawa's reaction		Free thiamine content (γ/dl)	
	Infant (months)	Mother (years)	Right	Left	Right	Left
1	3	22	2	1	5	15
2	7	30	1	1	15	11
3	4	28	2	2	7.5	10.5
4	7	26	2	2	7.5	7.5
5	15	40	2	2	7.5	5

TABLE II

Free Thiamine Content of Human Milk with Completely or Almost Completely Negative Arakawa's Reaction

No. of specimen	Age		Arakawa's reaction		Free thiamine content (γ/dl)	
	Infant (months)	Mother (years)	Right	Left	Right	Left
1	12	24	13	6	5	2.5
2	11	28	11	7	10	10
3	6	26	8	9	5	0
4	5	32	13	6	10	10
5	13	26	13	6	2.5	2.5
6	8	23	10	10	—	5
7	8	28	2	15	7.3	5
8	6	29	3	7	7.5	7.5
9	4	34	6	1	10	2.5
10	4	39	6	2	5	5
11	16	29	6	2	5	5
12	6	32	3	2	5	5
13	3	33	6	6	7.5	5
14	5	23	6	3	7.5	7.5
15	15	35	6	6	7.5	5
16	4	30	6	6	5	5
17	5	22	6	5	5	—
18	6	29	2	5	5	5

TABLE III

Free Thiamine Content of Human Milk with the Worse Intermediate Arakawa's Reaction

No. of specimen	Age		Arakawa's reaction		Free thiamine content (γ/dl)	
	Infant (months)	Mother (years)	Right	Left	Right	Left
1	11	28	39	39	0	0
2	7	27	23	14	2.5	2.5
3	6	21	2	24	—	2.5
4	20	24	28	44	2.5	2.5
5	1	27	24	44	2.5	2.5
6	9	40	24	24	0	2.5
7	12	27	20	44	2.5	2.5
8	6	31	48	28	2.5	2.5
9	18	26	32	32	5	2.5
10	1	24	24	32	0	0
11	11	37	23	13	2.5	0
12	14	32	47	58	5	5
13	2	32	39	39	2.5	—
14	3	25	31	28	2.5	0
15	13	31	24	24	2.5	2.5
16	4	29	20	20	2.5	2.5
17	2	43	42	22	5	—
18	12	28	22	36	—	7.3
19	2	21	43	34	2.5	—
20	7	24	10	44	—	2.5
21	12	22	28	42	2.5	2.5
22	20	30	36	30	5	5
23	15	42	40	—	12.5	—
24	7	38	31	47	10	—
25	20	32	20	40	10	5
26	3	23	46	28	5	5
27	5	29	15	15	2.5	2.5
28	2	26	13	13	5	5
29	13	24	20	10	—	5

It goes without saying that all the reagents used should be free of fluorescence.

Results and Remarks

The content of human milk in free thiamine was determined in 130 specimens of 72 lactants and the following results were obtained.

1. The content of human milk in free thiamine was 0–15 γ/dl.

2. The relation between the intensity of Arakawa's reaction and the free thiamine content of human milk is given in Tables I–VII.

a) The free thiamine content of milk with strongly or normally positive Arakawa's reaction was 9.2 γ/dl on an average (the maximum 15 γ/dl and the minimum 5 γ/dl), that of the better intermediate reaction 5.8 γ/dl on an average (the maximum 10 γ/dl, the minimum 2.5 γ/dl) that of the worse intermediate reaction 3.4 γ/dl (the maximum 12.5 γ/dl, the minimum 0 γ/dl), while the average thiamine content was only 3.1 γ/dl (the maximum

TABLE IV

Free Thiamine Content of Human Milk with Completely or Almost Completely Negative Arakawa's Reaction

No. of specimen	Age		Arakawa's reaction		Free thiamine content (γ/dl)	
	Infant (months)	Mother (years)	Right	Left	Right	Left
1	3	33	48	47	2.5	2.5
2	13	26	46	48	2.5	0
3	6	26	46	48	2.5	2.5
4	17	39	46	46	5	5
5	7	32	47	47	2.5	2.5
6	14	32	47	44	2.5	0
7	12	34	43	48	2.5	2.5
3	1	35	48	47	2.5	2.5
9	8	27	48	44	—	2.5
10	1	27	48	48	2.5	2.5
11	7	26	48	48	5	5
12	3	25	48	48	2.5	—
13	2	31	43	46	2.5	—
14	1	30	48	48	2.5	0
15	1	27	48	48	0	0
16	4	26	44	44	2.5	2.5
17	14	28	48	48	5	5
18	12	30	44	48	2.5	2.5
19	5	35	48	48	12.5	12.5
20	2	32	48	48	2.5	2.5

TABLE V

Human Milk Content of Free Thiamine and Arakawa's Reaction of 130 Milk Specimens

Groups of Arakawa's reaction	Number of specimens	Free thiamine (γ/dl)		
		Average	Maximum	Minimum
Strongly or normally positive	10	9.5	15	5
Better intermediate	34	5.8	10	2.5
Worse intermediate	49	3.4	12.5	0
Completely or almost completely negative	37	3.1	12.5	0

12.5, the minimum 0 γ/dl) in the case of the completely or almost completely negative Arakawa's reaction (Cf. Table V).

b) The free thiamine content of 80% of milk specimens of strongly or normally Arakawa's reaction fluctuated between 7.5 γ/dl (and15 γ/dl, while that of 78.4% (or about 80%) of milk specimens of completely or almost completely negative fluctuated between 0 γ/dl and 2.5 γ/dl (Cf. Table VI).

My own results of free thiamine content agree generally with those

TABLE VI

Distribution of 130 Milk Specimens according to Free Thiamine Content and to Arakawa's Reaction

Free thiamine (γ/dl)	Groups of lactants according to Arakawa's reaction			
	Strongly or normamally positive	Better intermediate	Worse intermediate	Completely or almost negative
15–7.5	8 (80%)	12 (35.3%)	4 (8.2%)	2 (5.4%)
5	2 (20 »)	17 (50 »)	12 (24.5 »)	6 (16.2 »)
2.5–0		5 (14.7 »)	33 (67.5 »)	29 (78.4 »)

published by Ts. Arakawa,[5] but my highest figures are slightly lower than the results by Neuweiler,[1] by Widenbauer and Heckler[3] or by Hirai and Ide.[2] As to the relation between Arakawa's reaction and free thiamine content of human milk, I have come to the same conclusion as Arakawa.[5]

It is to emphasize here that in case of thiamine determination of human milk, it is all important to exclude non-specific substances giving fluorescence so completely as is possible. Therefore it is very difficult or it is almost impossible in case of Arakawa-negative human milk to try to obtain the fluorescence only specific to thiamine, if the method of Neuweiler, Widenbauer and Heckler or Hirai and Ide' is used.

CONCLUSIONS

1. Using my own modification of the thiochrome method, I determined the free thiamine content of human milk (colostrum excluded).

2. Human milk positive to Arakawa's reaction was of far greater content in free thiamine than that negative to the reaction. Specimens of human milk with normal, weak and negative Arakawa's reaction showed the following average figures of free thiamine: 9.2 γ/dl, 7.4 γ/dl and 3.1 γ/dl respectively.

This work was carried out by a Grant from the Foundation for Scientific Research of the Ministry of Education. Prof. Akira Sato.

This work was completed in 1941 when he left our Laboratory for good, I shall make a few remarks on this work taking into consideration the paper by Roderuck, Williams and Macy (1945) in a later issue of this Journal. Prof. Akira Sato.

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Further Report on the Relation between Arakawa's Reaction and Thiamine Content of Human Milk

Part 2. Bound Thiamine of Human Milk*

227th Report of the Peroxidase Reatcion

(174th Human Milk Study)

By

Takeshi Naito
(内藤 武)

(From the Department of Pediatrics, Faculty of Medicine
Tohoku University, Sendai. Director: Prof. A. Sato)

(Received for publication, November 16, 1951)

Introduction

In a preceding paper[1] of mine, I have reported the relation between Arakawa's reaction and free thiamine content of human milk. The cocarboxylase identified as thiamine-pyrophosphate by Lohmann and Shuster[2] and the thiamine monophosphate which has the function of thiamine like the pyrophosphate, cannot be determined by the usual thiochrome method of thiamine assay, because[3-6] the oxidized product giving fluorescence will not, as is the case with unbound thiamine, go over into the isobutanol layer.

Widenbauer and Krüger[7] estimated thiamine demand of nurslings and presumed the occurrence of cocarboxylase in human milk, because thiamine demand per 100 calories of food of breast-fed infants would otherwise have been exceedingly lower than that of bottle-fed ones. They were, however, unable to identify the cocarboxylase at all.

In 1939 Ts. Arakawa[8-9] in our Laboratory asserted (or held for highly probable) by way of experiment the occurrence of cocarboxylase in human milk, and also that in human milk positive to Arakawa's reaction the coferment was contained in a far greater amount that in Arakawa-negative milk. Actually he performed the decarboxylation of pyruric acid by the alkaliwashed yeast and saw an increase of liberated carbonic acid.

Now the thiamine bound with phosphoric acid is split by phosphatase which is contained in the kidney,[10] Takadiastase,[11-16] yeast[17] or diastase[18]

* This was completed in 1944 and preliminarily shown at the Professors' Conference March 4, 1945.

(Merck), and the free thiamine is then obtained. I made use of the phosphate splitting action of Takadiastase (Sankyo) in order to utilize the thiochrome method for thiamine assay.

In the present paper (Part 2) I desire to report the relation between Arakawa's reaction and thiamine, esp. bound thiamine of human milk.

Experimental

Materials

Mothers of healthy infants and those breast-fed infants who visited our-patient Department were made objects of investigation. Lactants who had been taking thiamine preparations were excluded. Mothers with a new pregnancy, with menstruation or with mastitis were also excluded. So were lactants within 3 weeks of delivery.

Method

1. Testing of Arakawa's reaction (Comp. Part 1 of the present treatise).

2. Determination of thiamine

Specimens of human milk were examined as soon as possible, within 3 hours at the latest.

A. Determination of free thiamine (Comp. Part 1 of the present treatise)

B. Determination of bound thiamine

a) Preparation of ferment solutions

Takadiastase (Sankyo) contains thiamine in various amounts, which must be removed beforehand.

Add 10 cc. of destilled water to 0.5 g of Takadiastase in a flask and adjust to pH 3.0 with diluted HCl. Add 0.2 g of acid clay, shake vigorously for 5 minutes and filter using Toyo-filter paper No. 131. By this method a clear ferment solution without thiamine can be obtained.

b) Preparation of test and control solutions

1.0 cc. of skimmed human milk is used. After the digestion with pepsin as is described for free thiamine (Cf. Part 1 of the present treatise), add 8 cc. of phosphate buffer pH 4.5 and 1.0 cc. of the ferment solution above described, and keep it in incubator at 38°C for 4 hours.

The following part of the procedure of determination is quite the same as in case of the determination of free thaimine.

After washing with ether-isobutanol mixture, the water layer is divided into 2 aliquot parts for the preparation of both the test and the control solutions. This is the method for determination of total thiamine.

c) Determination of bound thiamine

Human milk contains no enzyme that can split phosphate from bound thiamine. Such an enzyme is not contained in pepsin (Merck) either, as the following result shows:—

Total thiamine.....7.5 γ/dl
Free thiamine.....Trace
} in one and the same specimen of milk

If pepsin (Merck) had contained such an enzyme as above mentioned, then both figures would have been equal to each other.

Bound thiamine will be calculated from:

Total thiamine—free thiamine

Results

118 milk specimens of 77 lactants were examined and the following results obtained.

1. The total thiamine of human milk was 2.5–25.5 γ/dl. The free thiamine was 0–12.5 γ/dl. and the bound thiamine 0–20 γ/dl.

2. The relation between the Arakawa reaction and the total, the free and the bound thiamine content of human milk is given in Tables I–V. As Table V shows, it is indubitable that in general the content of human milk in the total, the free and the bound are far greater in Arakawa-positive milk. Averagely, the bound thiamine was of somewhat higher content than the free thiamine, though in some cases the reverse was true.

Remarks

Soon after the discovery of Lohmann and Shuster[2] who had identified the pyrophosphoric acid ester of thiamine as socarboxylase, Lipmann[21] showed that cocarboxylase was necessary for the oxidation of pyruvic acid by acetone-washed B. delbrücki. Barron and Lymann[22] found that in animal tissue and bacteria the pyrophosphate of thiamine was effective as catalyser not only for decarboxylation and aerobic oxidation of pyruvic acid, but also for an aerobic dismutation of it, further probably for many other reactions,[23] e.g. synthesis of carbohydrate and citric acid, where pyruvic acid was a reacting substance. And it was shown by many authors that thiamine could be phosphorylated immediately by animal tissue and yeast. And if the tissue can phosphorylate, then thiamine can perform the functions of cocarboxylase. Lohmann and Schuster's theory attributing all biologic functions of thiamine to the effect of pyrophosphoric ester has become very probable. Besides, thiamine and its monophosphate ester possess[28] an encouraging effect for the decarboxylation of pyruvic acid, which process must be performed by cocarboxylase.

Now it is indubitable that human milk contains generally a larger amount the bound form of thiamine than of the free form, and that both forms of the vitamin are contained in a far larger amount in the Arakawa-positive milk than in the Arakawa-negative milk.

Asari[29] did not recognize any relation between the bound and the free forms and again any relation to the Arakawa reaction. Her method of determination was a modification of thiochrome method. It was ap-

Table I

Thiamine Content of Strongly or Normally Arakawa-positive Milk

No.	Date	Age of mother / infants	R=Right L=Left	Arakawa's reaction	Thiamine		
					Total	Free	Bound
1	22.I	26y	R	2	20	7.5	12.5
		7m	L	2	27.5	7.5	20
2	21.II	40y	R	2	17.5	7.5	10
		20m	L	2	17.5	5	12.5
3	30.IV	25y	R	2	—	—	—
		2m	L	2	17.5	10	7.5
4	26.VII	25y	R	2	10	2.5	7.5
		7m	L	1	—	—	—
5	5.VIII	29y	R	1	12.5	7.5	5
		8m	L	1	12.5	7.5	5
6	5. »	25y	R	1	17.5	10	7.5
		9m	L	1	20	7.5	12.5
7	5. »	24y	R	1	15	12.5	2.5
		7m	L	1	—	—	—
8	8. »	28y	R	2	12.5	7.5	5
		8m	L	2	15	7.5	7.5
9	8. »	22y	R	1	—	—	—
		10m	L	1	20	12.5	7.5
10	8. »	27y	R	1	25	10	15
		9m	L	1	25	10	15
11	8. »	30y	R	2	12.5	5	7.5
		6m	L	2	—	—	—
12	14. »	31y	R	1	15	7.5	7.5
		6m	L	1	15	7.5	7.5
13	14. »	32y	R	2	17.5	12.5	5
		6m	L	2	—	—	—
14	14. »	35y	R	2	22.5	12.5	10
		5m	L	2	22.5	12.5	10
15	14. »	40y	R	2	—	—	—
		7m	L	2	17.5	7.5	10
16	14. »	30y	R	1	22.5	7.5	15
		13m	L	1	22.5	7.5	15
17	17. »	29y	R	1	—	—	—
		5m	L	1	15	5	10
18	17. »	32y	R	2	12.5	2.5	10
		8m	L	1	—	—	—
19	17. »	40y	R	1	—	—	—
		6m	L	2	17.5	12.5	5
20	17. »	43y	R	2	—	—	—
		14m	L	1	20	7.5	12.5
21	17. »	30y	R	2	12.5	7.5	5
		4m	L	1	—	—	—

Table II

Thiamine Content of Completely or Almost Completely Arakawa-negative Milk

No.	Date	Age of mother / infants	R=Right L=Left	Arakawa's reaction	Thiamine		
					Total	Free	Bound
1	3.XII '41	22y	R	6	15	5	10
		5m	L	5	—	—	—
2	3. »	23y	R	10	—	—	—
		8m	L	10	20	5	15
3	17. »	26y	R	13	20	2.5	17.5
		13m	L	6	20	2.5	17.5
4	23. »	28y	R	2	22.5	7.5	15
		8m	L	15	20	5	15
5	26.I	29y	R	3	15	7.5	7.5
		6m	L	7	15	7.5	7.5
6	6.II	29y	R	2	17.5	5	12.5
		6m	L	10	20	5	15
7	12.V	33y	R	10	15	7.5	7.5
		4m	L	10	—	—	—
8	14. »	24y	R	10	15	10	5
		6m	L	10	—	—	—
9	22. »	26y	R	10	10	2.5	7.5
		11m	L	2	15	7.5	7.5
10	11.VI	25y	R	2	17.5	5	12.5
		12m	L	10	17.5	5	12.5
11	15. »	58y	R	8	—	—	—
		5m	L	2	15	7.5	7.5
12	29. »	28y	R	13	—	—	—
		9m	L	7	12.5	7.5	5
13	3.VII	34y	R	8	12.5	10	2.5
		6m	L	8	12.5	10	2.5
14	15. »	25y	R	5	12.5	7.5	5
		1m	L	1	12.5	7.5	5
15	22. »	24y	R	10	10	2.5	7.5
		6m	L	10	—	—	—
16	29. »	25y	R	10	15	10	5
		4m	L	2	15	10	5

TABLE III

Thiamine Content of Milk of Worse Intermediate Arakawa's Reaction

No.	Date	Age of mother infants	R=Right L=Light	Arakawa's reaction	Thiamine		
					Total	Free	Bound
1	24.XI '41	29y	R	20	7.5	2.5	5
		4m	L	20	7.5	2.5	5
2	15.XII	43y	R	36	12.5	5	7.5
		2m	L	22	—	—	—
3	18. »	28y	R	22	—	—	—
		12m	L	36	15	7.5	7.5
4	19. »	21y	R	43	15	2.5	12.5
		2m	L	34	—	—	—
5	21. »	24y	R	20	—	—	—
		13m	L	10	15	5	10
6	22. »	24y	R	10	—	—	—
		7m	L	44	17.5	2.5	15
7	24. »	22y	R	28	17.5	2.5	15
		12m	L	42	17.5	2.5	15
8	9. I '42	30y	R	36	15	5	10
		20m	L	30	17.5	5	12.5
9	13. »	42y	R	—	—	—	—
		15m	L	40	12.5	12.5	0
10	16. »	38y	R	32	12.5	10	2.5
		7m	L	47	—	—	—
11	25. II	32y	R	20	15	10	5
		20m	L	40	15	5	10
12	28. III	23y	R	46	12.5	5	7.5
		3m	L	28	15	5	10
13	7. IV	28y	R	13	12.5	5	7.5
		3m	L	17	12.5	5	7.5
14	10. »	42y	R	13	20	7.5	12.5
		6m	L	17	20	7.5	12.5
15	7. VII	27y	R	24	—	—	—
		4m	L	10	12.5	7.5	5
16	21. »	39y	R	28	—	—	—
		3m	L	40	7.5	7.5	0
17	22. »	28y	R	36	—	—	—
		18m	L	40	12.5	10	2.5
18	24. »	27y	R	22	7.5	7.5	0
		4m	L	36	10	7.5	2.5
19	3.VIII	39y	R	38	—	—	—
		3m	L	43	10	7.5	2.5

TABLE IV

Thiamine Content of Completely or Almost Completely Arakawa-Negative Milk

No.	Date	Age of mother infants	R=Right L=Left	Arakawa's reaction	Thiamine		
					Total	Free	Bound
1	27.XI '41	27y	R	48	5	2.5	2.5
		1m	L	48	5.5	2.5	5
2	2.XII	26y	R	48	7.5	5	2.5
		7m	L	48	10	5	5
3	4. »	25y	R	48	10	2.5	7.5
		3m	L	48	—	—	—
4	16. »	31y	R	42	10	2.5	7.5
		2m	L	46	—	—	—
5	20. I '42	30y	R	48	12.5	2.5	10
		1m	L	48	12.5	0	12.5
6	23. »	27y	R	48	7.5	0	7.5
		1m	L	48	2.5	0	2.5
7	24. »	26y	R	44	15	2.5	12.5
		4m	L	44	15	2.5	12.5
8	4. II	28y	R	48	12.5	5	7.5
		14m	L	48	12.5	5	7.5
9	19. »	30y	R	44	2.5	2.5	0
		12m	L	48	2.5	2.5	0
10	23. »	35y	R	48	12.5	12.5	0
		5m	L	48	12.5	12.5	0
11	26. »	32y	R	48	7.5	2.5	5
		2m	L	48	7.5	2.5	5
12	15.III	31y	R	44	7.5	5	2.5
		4m	L	48	7.5	5	2.5
13	17. »	30y	R	44	5	5	0
		2m	L	44	5	5	0
14	18. »	28y	R	48	2.5	2.5	0
		8m	L	48	2.5	2.5	0
15	9.IV	27y	R	48	12.5	5	7.5
		2m	L	48	12.5	5	7.5
16	16. »	23y	R	48	10	7.5	2.5
		2m	L	48	—	—	—
17	20. »	31y	R	48	12.5	7.5	5
		6m	L	48	—	—	—
18	22. »	30y	R	48	12.5	7.5	5
		14m	L	—	—	—	—
19	27. »	29y	R	44	12.5	2.5	10
		2m	L	48	12.5	2.5	10
20	30. »	25y	R	48	—	—	—
		2m	L	48	12.5	2.5	10
21	24. »	28y	R	48	10	0	10
		2m	L	48	10	0	10

TABLE V

Relation between Arakawa's Reaction and Thiamine Content of Human Milk

Arakawa's reaction	No. of specimens	Thiamine on average (γ/dl)*		
		Total	Free	Bound
Strongly or normally positive	30	17.7	8.3	9.4
Better intermediate	25	15.7	6.5	9.2
Worse intermediate	27	13.5	6.0	7.5
Completely or almost Completely negative	36	9.2	3.8	5.4

* Micrograms per 100 cc.

parently a good method, but she committed an obvious error: she added to milk specimens too large an amount of thiamine as ten times the pre-existing vitamin or more. Suppose a specimen contained 5 γ/dl of thiamine. To 1.0 cc. of milk (i.e. with 0.05 γ thiamine in it) she added 0.5 γ of pure thiamine and was to determine 0.55 γ in total. In such a macro method as Volhard's determination method for chlorine, for instance, one could try such an addition and obtain still a fairly correct answer. With such a difficult method as the thiochrome method for human milk one should not work in such a way; besides, her classification of Arakawa's reaction was not good. I shall not go into details here, but refer to an article[31] (to be published hereafter).

Now, human milk negative to Arakawa's reaction is a milk from thiamine-deficient body, so an abnormal or bad milk, as the numerous papers (on the Peroxidase Reaction) from our Laboratory have shown it. My own result has convinced us again, that Arakawa-positive milk is generally a good human milk.

CONCLUSIONS

The content of human milk in the total and free forms of thiamine was determined at the same time by my own modification of the thiochrome method and the bound form was calculated. The result is as follows:—

1. The bound form of thiamine was averagely contained in a larger amount than the free form.
2. The total, free and bound forms of thiamine was generally of a far larger content in Arakawa-positive milk than in the Arakawa-negative.
3. It will thus be shown that Arakawa-positive milk is generally a good milk.

This work was carried out by a Grant from the Foundation for Promotion of Scientific Research of the Ministry of Education. Prof. Akira Sato.

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The Tohoku Journal of Experimental Medicine, Vol. 56, Nos. 1-2, 1952

Thiamine in Human Milk. As to the Question of Priority of Identifying Bound Thiamine in It

By

Akira Sato

(佐藤　彰)

(From the Department of Pediatrics, Faculty of Medicine, Tohoku University, Sendai. Director: Prof. Akira Sato)

(Received for publication, November 16, 1951)

It has become necessary to publish here a translation of the abstract of my speech[1] dilivered on June 26, 1942, because those who will read the papers of Ts. Arakawa[2] and T. Naito[3-4] published in this Issue of the Journal may wonder how we did ever know the occurrence of bound thiamine in human milk as early as 1942. In reality, we began to suspect it as early as 1940 (see below).

My paper[1] was read in the 124th ordinary meeting of the Tohoku Medical Society. The following is a translation of the abstract printed in Japanese.

Vitamin B_1 in Human Milk

At first the question[5]: If two specimens of human milk—Milk A and Milk B—are equal in amount of vitamin B_1, then are they of equal biological significance, as far as this vitamin is concerned?—is treated.

Then,—

"Dr. Asari published in April this year the relation between Arakawa's reaction and vitamin B_1. I have seen this Report only recently, and I have to say about the bound vitamin B_1 in human milk, as Asari paper also relates of it.

"Naito,[3] of our Clinic, published 'the relation between Arakawa's reaction and vitamine B_1 of human milk' in the 47th General Meeting of the Japanese Pediatric Society, held 1942 in Tokyo. He then limited his report to the free vitamine B_1 in human milk (he had only 8 minutes allotted to him), but he had with him his own data of bound vitamin B_1 in a typewritten manuscript,[4] because he had expected that some one would probably ask him whether he had also investigated it in his materials. But nobody asked him such a question.

"Naito,[4] however, was not the first one in our Clinic that had ever investigated bound vitamin B_1 in human milk. Dr. Arakawa,[2] as the first in our Clinic, began to consider as probable the existence of it in spite of the opinion to the contrary in the thitherto literature.

"—It was about the time from the end of July to the beginning of August 1940. And until the beginning of August 1941 he had attacked the problem from three different angles of investigation, and continued to study it until he left our Clinic on July 27, 1942 to serve as Surgeon in the Army.

"Naito[4] took over the studies and was thus the second investigator of the problem in our Clinic."

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Biochemical Studies on Carbohydrates

CLIV. Mucosin

Fourth Report

By

Tyuiti Isikawa
(石川 忠一)

(From the Medico-chemical Institute, Tohoku University, Sendai;
Director: Prof. H. Masamune)

(Received for publication, November 16, 1951)

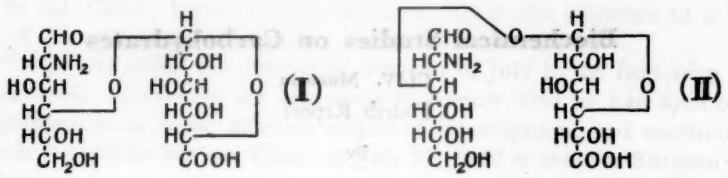
Jeanloz and Forchielli[1] claimed that, in mucoitin (hyaluronic acid) glucuronic acid combines 3-C of the adjacent acetylglucosamine glucosidically, standing on the basis that mucoitin had used 0.2 to 0.3 mole of periodate per mole, releasing 0.1 mole of formic acid, and a scission product, partially deacetylated by alkali, of the polysaccharide 0.8 to 1.0 mole of the oxidant without evolution of ammonia. Those quantities of periodate reduced pointed to the fact that oxidation had occurred in the chain extremities alone of the native polysaccharide and the disintegration product, and it was reasoned therefrom that the monosaccharide components both possess a pyranose structure.

On the other hand, Kurt H. Meyer and Fellig[2] insisted the linkage in question to be 1–4, because they obtained, by permethylation of mucoitin followed by methanolysis, a dimethylmethylglucosaminide which did not react with periodic acid. To draw the conclusion, they hopothesized the pyranose structure of the hexose units.

Both of the two groups of investigators assumed β-glucuronide- and β-glucosaminide-union from the negative optical rotation of mucoitin.

In order to settle the dispute the writer took N-acetylmucosin methylester[3] as a material and subjected to periodic acid oxidation under the conditions preferred by Yosizawa[4] and also estimated the amount of formaldehyde produced according to Masamune and Yosizawa.[5] The substance reduced 2.96 moles (99% of theor.) of the oxidant per mole and released 0.93 mole (93% of theor.) of the aldehyde. The result indicates that either glucosamine-4-β-glucuronide (1,5) (I) or glucosamine-3-β-glucuronide (1,4) (II) is the configuration to be assigned to mucosin. The enzymological evidence that mucosin is a β-glucuronide has been recorded already in the Third Report. An experiment as to whether

glucuronic acid exists in a pyranose structure or in a furanose structure is now being in progress.



Experimental

N-acetylmucosin methylester was prepared as described in the Second Report.[3] 37.54 mg. of it were dissolved in 7 cc. of water and after addition of 3.5 cc. of 0.2595 *M* HIO_4, maintained at 20°C, until the change of optical rotation ceased (About 6 hours required). Thereupon 4.5 cc. portions of the mixture were pipetted out for estimation of HPO_4 according to Yosizawa[4] and of formaldehyde according to Masamune and Yosizawa[5]. 0.278×10^{-3} mole of HIO_4 remained unreduced and 10.2 mg. of formaldehyde-dimedon compound [m. p. (cor.) 189–190°C] were separated. Calculated from the values, the substance consumed 2.96 $\left[=(0.2595\times3.5\times4.5/10.5-0.278)\times10^{-3}\times\frac{429}{37.54\times4.5/10.5}\times10^{3}\right]$ moles of HIO_4 per mole and gave 0.93 $\left[=\left(10.2\times10^{-3}\times\frac{429}{37.54\times4.5/10.5}\times10^{3}\right)/292\right]$ mole of the aldehyde.

Through the Grant Committee of the Science Council was given a grant from the Education Department in aid to us. H. M.

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Biochemical Studies on Carbohydrates

CLV. On Mucosin and Mucoitin

By

Hajime Masamune and **Zensaku Yosizawa**
(正宗 一) (吉澤善作)

(From the Medico-chemical Institute, Tohoku University, Sendai)

(Received for publication, November 16, 1951)

According to Isikawa[1], mucosin, the disaccharide unit of mucoitin, is either glucosamine-4-β-glucuronide (1,5) or glucosamine-3-β-glucuronide (1,4). Experimentation with periodic acid on mucoitin, the native polysaccharide, may provide a key to decide which of the two possibilities above is the truth. So from human umbilical cords was prepared pure mucoitin, whose molecular weight was calculated at some 9300 from iodine use. It consumed approximately 3.0 moles of periodic acid per mole like mucosin. Since Osaki and Hasegawa[2] revealed that acetylglucosamine and glucuronic acid stand at the reducing and non-reducing ends respectively of the molecule, the amount of periodic acid reduced indicates that the extremities alone did react with the oxidant. This simultaneously supports the opinion that the chain is an unbranched. The inertness of the internal part suggests a pyranose structure of acetylglucosamine units in this part, because, if these units be a furanose, they would use 1 mole of the oxidant per unit. The glucuronic acid units can take either a furanose or a pyranose structure, according to the position bound glucosidically by the adjacent acetylglucosamine, as periodic acid is not reduced by them at all if 2- or 3-C of the furanose or 3-C of the pyranose takes part in the linkage. Thus the present experiment led us to no conclusion as regards the oxide ring structure of glucuronic acid in mucosin, although, it established the pyranose structure of glucosamine units in mucoitin.

Experimental

Material

Mucoitin. 0.7 g. of its acid barium salt after Isikawa[3] was taken up in 10 cc. of water, a trace of an insoluble part centrifuged off and the supernatant was freed from barium by cautious addition of H_2SO_4. The filtrate and washings were distilled together to about 10 cc. and precipi-

tated with a mixture of 3 volumes of abs. alcohol and 5 volumes of dry ether. The gelationous deposit was centrifuged, washed with dry ether and dried. 550 mg. was a white powder yielded. On reprecipitation from water were given 420 mg. Goldschmiedt strongly positive, the indirect Osaki-Turumi positive, Molisch negative. After drying to a constant weight (in vacuo over H_2SO_4) it was subjected to analysis and measurement of optical rotation, which gave figures as follow: N (micro Kjeldahl) 3.2%; glucosamine 42.3%; glucuronic acid 45.8% (indole method: 46.0%, orcinol method: 45.6%); acetyl 10.1%; (equivalent ratios: 0.98:1.0:1.0:1.0). Ash 0.34%. Mol. wt. from iodine use (Macleod-Robison) 9249 (20 mg. of the substance used 0.23 cc. of 0.02 *N* iodine solution). $[\alpha]_D^{20}=\frac{-0.65\times 100}{1.0\times 1}=-65.0°$ in water.

Periodic Acid Oxidation

The procedure of Yosizawa[4] was employed. 200 mg. of the material were dissolved in 10 cc. of water and immediately after addition of an equal volume of 0.0249 *M* HIO_4, placed in a thermostat at 20°C. The change of optical rotation stopped in about 3.5 hours, when 10 cc. were pipetted out to determine the quantity of HIO_4 unreduced. The remaining HIO_4 in 10 cc. of the mixture was 0.0907×10^{-3} mole and consequently 0.0338×10^{-3}[$=(0.0249\times 10\times 10/20-0.0907)\times 10^{-3}$] mole was used, which corresponds to 3.13 ($=0.338\times 10^{-3}\times 9249/(200\times 10/20\times 10^{-3})$) mole for 1 mole of the mucoitin.

Summary

The consumption of HIO_4 by mucoitin was measured and a proof was brought forward that the internal glucosamine units of the acid polysaccharide chain are a pyranose, but none regarding the oxide ring structure of glucuronic acid in mucosin.

The costs of this work were defrayed by a grant from the Education Department. H. Masamune.

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Note at proof-reading: Articles below have been read, the first in a journal number and the second and third in a reprint received after contribution of this and just foregoing papers.

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Biochemical Studies on Carbohydrates

CLVI. On the Sugar Components of the Hexosamine-containing Carbohydrates from Gastric Cancers, Normal Human Gastric Mucosa and Human Liver and of the Glacial Acetic Acid-soluble Proteins from those Tissues as well as a Metastasis in Liver of Gastric Cancer

By

Hajime Masamune, Zensaku Yosizawa, Kiyota Oh-Uti,
(正宗 一) (吉澤善作) (大内清太)
Yasuaki Matuda and **Azuma Masukawa**
(松田順皓) (益川 東)

(From the Medico-chemical Institute, Tohoku University, Sendai)

(Received for publication, November 16, 1951)

We have mentioned in previous articles[1)2)3)] that group carbohydrates and a group lipoid of gastric cancers were less anti-isohemoagglutinative than the corresponding substances in normal gastric mucosa despite of the apparently resembling compositions. Furthermore the carbohydrates from gastric cancers of persons belonging to different blood groups had nearly equal optical rotations, notwithstanding that Group B- and Group O-carbohydrate from gastric mucosa were about 23° more levorotatory than Group A carbohydrate from the same source. The findings pointed to the conclusion that those constituents of cells of stomach cancers[4)] are "biologically immature." Whether or not all other high-molecular constituents of carcinomata are immature too is now under investigation. It should be also remarked that animal tissues or organs show each a peculiar feature in the make up, diverging in the high-molecular cell constituents in qualitative as well as quantitative composition, as is obvious from our works, hitherto reported in this Journal and forthcoming. Therefore, it might be said generally that primary carcinomata are built of high-molecular substances composed similar to those of their site and, hence, they differ from one another according to the tissue, where they proliferate, not only histologically but also chemically.[5)]

As to secondary tumors, we[3)6)7)] have noticed by quantitative analysis that a liver metastasis of gastric cancer contained a group lipoid and a group carbohydrate identical with the corresponding constituents of the primary tumor, but the glacial acetic acid-soluble proteins in the former growth diverged from those in the latter. Namely, if again generali-

zation be allowed, it appears plausible that, in metastasis, some of the high-molecular cell constituents of tumors undergo a variation which, however, is not replacement with the corresponding fraction of the cells in the organ where the secondary growth occurs.

To ensure the views, the materials in the heading, mostly left unexhausted in former studies, were hydrolyzed and subjected to paper partition chromatography to identify their sugar components. The spots developed on paper strips were localized at positions of chondrosamine, glucosamine, galactose, mannose and a methylpentose (probable L-fucose) in the group carbohydrates (Group A, B and O) from gastric cancers (I, II, III) and the one (Group A) from gastric mucosa (IV), and at those of chondrosamine, galactose and a methylpentose (probable L-fucose) in the glacial acetic acid-soluble protein fraction of gastric cancers (V) and gastric mucosa (VI), which both belonged to Group A.

By the hydrolysate of the glacial acetic acid-soluble protein fraction of the liver metastasis (VII) were given the spots of chondrosamine, glucosamine, galactose, mannose and a methylpentose (probable L-fucose), but by that of the corresponding fraction of liver (IX) was given a spot of a pentose in addition to those of the above sugars. The group carbohydrate (low Group-active) of liver (VIII) was revealed by the spots obtained to contain chondrosamine, glucosamine, galactose, a pentose and a methylpentose (probable L-fucose). A small spot of glucose was also shown, which is supposedly due to a contaminant (Cf. Matuda[8]).

The coresponding fractions of gastric cancer and gastric mucosa, which had been already considered similar in composition[1][2] were thus found in effect to possess the same kinds of sugar.

The group carbohydrate in the liver metastasis could not be examined because of shortage of substance, but it is suspected to give equal chromatograms as that from the original growth, to which it was similar in the quantitative analyses of the components (See Matuda[9]), if analogized from the quantitative-analytical relation between the group carbohydrates from gastric mucosa and gastric cancer, whose chromatograms suggested same sugars, and, moreover, to be different from the group carbohydrate in liver because of the quantitative-analytical and serological-quantitative (anti-isohemoagglutinative) differences of the latter from that of gastric mucosa. The glacial acetic acid-soluble protein fraction of the metastasis has been proved dissimilar[9] not only from that of the original growth but also from that of normal liver.

The present findings support the conceptions hitherto held by us.

To add in passing, Group A carbohydrate from the stomach mucosa of pig (X) also gave chromatograms quite resembling those of the group carbohydrates from human gastric mucosa.

Experimental

Materials

1) Group A-, B- and O-carbohydrate from gastric cancers (Will be referred to as **I, II** and **III**): the preparations of Oh-Uti[1].

2) Group A carbohydrate from human gastric mucosa (**IV**). Prepared by a similar procedure to the one Oh-Uti[1] had applied to gastric cancers. N 4.2%; hexosamines 40.3%, non-amino sugars as galactose 41.5% (indole method: 42.2%, orcinol method: 40.7%); ash 1.5%. Anti-isohemoagglutinative dilution $1:10^6$.

3) The glacial acetic acid-soluble protein fraction from the gastric cancers of persons belonging to Group A in 1) (**V**): the preparation of Oh-Uti[1].

4) The glacial acetic acid-soluble protein fraction from gastric mucosa of persons belonging to Group A (**VI**): the preparation by Masukawa.[10]

5) The glacial acetic acid-soluble protein fraction from a metastasis in liver of a gastric cancer (blood group of the patient, B) (**VII**): The preparation by Matuda.[9]

6) The group carbohydrate from livers of persons belonging to Group A (**VIII**): the preparation by Matuda.[8]

7) The glacial acetic acid-soluble protein fraction from livers of persons belonging to Group A (**IX**): the preparation of Matuda.[7]

8) The Group A carbohydrate of pig stomach-mucosa (**X**). Prepared by Oh-Uti as the corresponding carbohydrates from gastric cancers. N 4.3%; hexosamines 41.1%; non-amino sugars as galactose 41.5% (indole method 42.0%, orcinol method 41.0%); ash 1.3%. Anti-isohemoagglutinative dilution $1:10^6$.

Chromatography

The technique, the one described by Yosizawa.[11]

To a weighed amount of the preparations were added 100 volumes of 1 *N* H_2SO_4.

Preparations	I	II	III	IV	V	VI	VII	VIII	IX	X
Amount taken (mg.)	20	15	20	30	50	50	100	40	50	30

Each of the solutions was heated in an ampoule (100°C, 5 hrs.) and after eliminating most of H_2SO_4, distilled in vacuo to about 1/2.5 the volume of the initial solution in the cases of the carbohydrates and to about 1 cc. in the cases of proteins. The volume of the condensates put on paper were the following:

Preparation	I	II	III	IV	V	VI	VII	VIII	IX	X
Volume put (cc.)	0.018	0.018	0.018	0.018	0.035	0.035	0.06	0.03	0.035	0.018

Solvents employed were *n*-butylacetate-pyridine-water (5:3:1) (duration of the test about 8.5 hrs.) and *n*-butylacetate-acetic acid-water (3:2:1) (duration of the test about 15.5 hrs.). Solvent fronts run 40 cm. Temperature, 19–20°C. Developer, $AgNO_3$-ammonia.

Results

The chromatograms obtained are reproduced diagrammatically in Figs. 1 and 2.

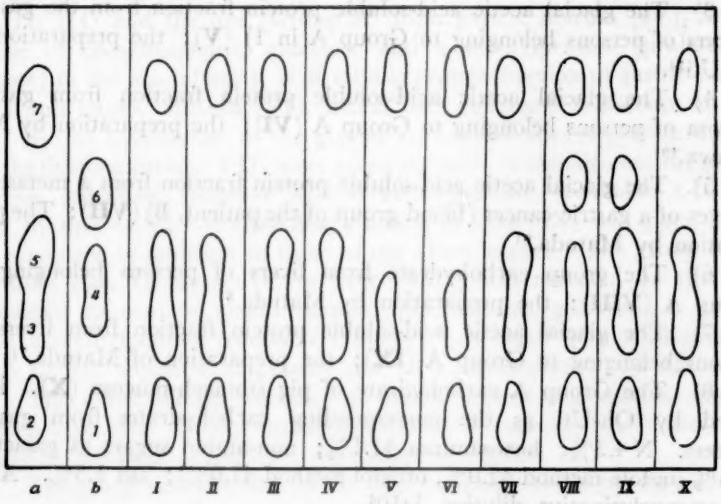

Fig. 1. Diagrammatic reproduction of the *n*-butylacetate-pyridine-water (5:3:1) chromatograms.
a and b: standards. I-X: the corresponding preparations (See the text).
1: chondrosamine sulfate, 2: glucosamine sulfate, 3: galactose, 4: glucose, 5: mannose, 6: arabinose, 7: rhamnose.

In Fig. 1 glucosamine and chondrosamine are not distinguished. The spots indicate sugars (+ contained, − none):

Preparation	I	II	III	IV	V	VI	VII	VIII	IX	X
Hexosamine	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	−	−	+	−	+	+
Glucose	−	−	−	−	−	−	−	+	−	−
Pentose	−	−	−	−	−	−	−	+	+	−
Methylpentose (probable L-fucose)	+	+	+	+	+	+	+	+	+	+

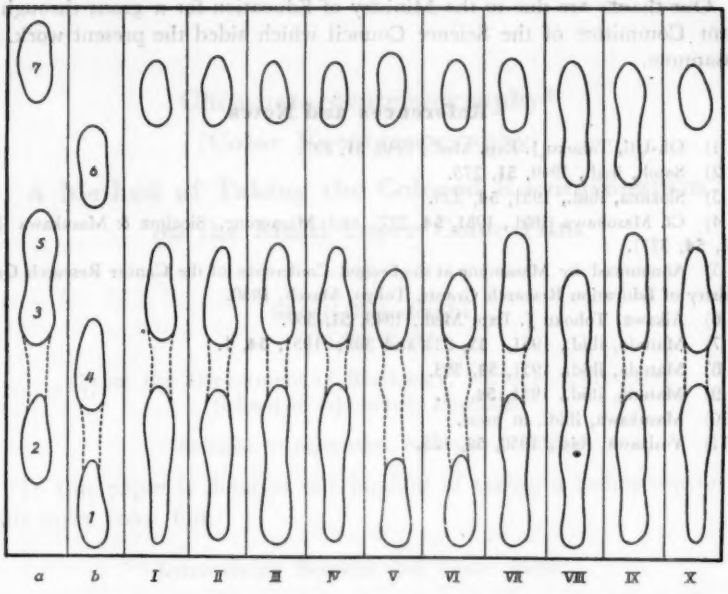

Fig. 2 Diagrammatic reproduction of the *n*-butylacetate-acetic acid-water (3:2:1) chromatograms.

a and b: standards. I-X: the corresponding preparations (See the text). 1: chondrosamine sulfate, 2: glucosamine sulfate, 3: galactose, 4: glucose, 5: mannose, 6: arabinose, 7: rhamnose.

The spots in Fig. 2 demonstrate the occurrence of sugars (+ contained, — none):

Preparation	I	II	III	IV	V	VI	VII	VIII	IX	X
Chondrosamine	+	+	+	+	+	+	+	+	+	+
Glucosamine	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	−	−	+	−	+	+
Glucose	−	−	−	−	−	−	−	+	−	−
Pentose	−	−	−	−	−	−	−	+	+	−
Methylpentose (probable L-fucose)	+	+	+	+	+	+	+	+	+	+

Conclusion

The sugar chromatograms obtained with the substances from human gastric mucosa, gastric cancers, human livers and a liver metastasis of a gastric cancer supported our opinions regarding the composition of high molecular constituents of cancer and its metastasis.

Our thanks are due to the Ministry of Education for a grant through the Grant Committee of the Science Council which aided the present work. H. Masamune.

References and Notes

1) Oh-Uti, Tohoku J. Exp. Med., 1949, **51**, 297.
2) Satoh, ibid., 1949, **51**, 275.
3) Siozima, ibid., 1951, **54**, 333.
4) Cf. Masukawa (ibid., 1951, **54**, 227) and Masamune, Siozima & Masukawa (ibid., 1951, **54**, 327).
5) Announced by Masamune at the Second Conference of the Cancer Research Group, Ministry of Education Research Grants, Tokyo, March, 1950.
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Chromatoroentgenography*
(Color Roentgenography)
A Method of Taking the Colored Roentgenogram on the Multi Layer Color Film

By

Shinji Takahashi
(高橋 信次)

(From the Department of Radiology, Hirosaki University, School of Medicine, Hirosaki)

(Received for publication, November 27, 1951)

In this paper is detailed our method of taking a radiogram on the multi layer color film.

Intensifying Screens and Color Film

Two sheets of intensifying screens had been prepared beforehand: Screen A and B. Screen A were plastered on its surface with the fluorescent substances of ZnS/Ag and CdS/Ag which were mixed homogenously in the ratio of 1 to 4. Irradiated by x-ray, this screen emitted red orange light. On Screen B were plastered the same fluorescent substances mixed in the reversed ratio of 4 to 1. This emitted blue green light at x-ray irradiation.

In our experiment was used the Fujicolor (or Fuji Multi Layer Color film).

Model Experiment

In order to see how the film would be colored by our method, we conducted the following model experiment:

Four square aluminium sheets of 1 mm thickness and a sheet of copper 0.5 mm thick were arranged in stack, so that the edge 1 cm wide of each sheet might be left uncovered by the one following. This stack of sheets was the model used. The experiment was processed in the dark room. The intensifying screen A, on which the model was mounted,

* Presented at 2nd the Hirosaki Radiologists' Meeting, March 4, 1951. The Chromatatoroentgenograms referred to in this paper were demonstrated at the 7th Meeting of the Tohoku Hokkaido Niigata Radiol. Society, Sept. 30, 1951.

was placed on top of the color film. Its first exposure was made under the following conditions: the tube terminal 150kV, tube current 1 ma, the copper filter 0.5 mm in thickness, the length of exposure one second, and the focus film distance 50 cm. After the exposure, Screen A alone was removed and in its place, the intensifying Screen B was put between the model and the film. The second exposure was performed with 37kV of tube terminal, 2 ma, without using the filter, for 15 seconds, at 25 cm of the focus film distance.

The roentgenogram taken in this manner shows the following:

With increase of the thickness of metal sheets stack, the x-ray image of the model changes in color as well as in color depth, from red to light redblue, light reddish blue, blue with very slight red hue, blue green and finally to dark blue green.

Human Body Experiment

In order to see whether this methed is worth clinical application and has any merit or not, we made the following test in chromatoroentgenography.

A case, revealing, in its normal roentgenogram, fibrous productive lesions with calcifications in the upper filed of the right lung, was radiographed in the same way as in the model experiment.

With Screen A the first exposure was made under 45kV, 150 ma, for 1 second, at 100 cm of the focus film distance. Immediately after this exposure, Screen A was replaced by B, which then was exposed under 72kV, 150 ma, for 0.4 seconds. The chromatoroentgenogram taken this time showed:

The healthy portion of pulmonary area is colored red. At the infraclavicular median region there is manifested a marked light redness of a deformation of coin size, which may be regarded as cavitation in old tuberculosis. In the remaining region of the lung are found a number of scattered nodulous blue images representing tuberculous foci of the fibrous and productive feature. The marked dark blue images in them may be accepted as calcifications.

In order to set this chromatoroentgenogram against the black and white roentgenogram, we took two ordinary roentgenograms of the same patient. The one was taken with soft x-ray, while the other was exposed to hard ray. In the former the foci of fibrous and productive feature were dominant in the image, but in the latter only calcifications and cavitation were clearly imaged.

The chromatoroentgenography was also applied to the recording of the motion of a working organ.

With Screen A, for instance, we exposed the expired stationary

diaphragma under 62kV, 150 ma, for 0.3 seconds, at 100 cm of the focus film distance, then, replacing Screen A with B, the inspired stationary diaphragma was exposed under the same voltage and miliamperage, but for 0.5 seconds. In the chromatoroentgenogram thus obtained we learned that the light blue band of diaphrama showed the amplitude of its movement for respiration.

We applied this method, furthermore, to stereography. The chromatostereoroentgenogram of a forearm was taken and studied.

Discussion

At the 2nd Meeting of the Hirosaki Roentgenologists' Society held on March 4, 1951, we published our Chromatoroentgenography (Color Rentgenography), preliminary report, for the first time.[1] At that occasion we stated this method might be adopted not only for the ordinary radiography, but also for the motion radiography and stereoradiography in the near future. It was also mentioned that there are two technical ways for applying this method. The first corresponds to the technicolor method. Several roentgenograms taken under different exposing conditions are to be dyed each with different kinds of color, and to be stacked for inspection. The second may be regarded as the color photography of the multi layer color system. Several roentgenological exposures are to be made upon a single color film under different exposing conditions. Here, however, the intensifying screen, which is to be replaced at every different exposure, plays the rôle of dye. The chromatoroentgenography should not be considered as a composition, but a synthesis of two roentgenograms which were exposed under different conditions. Due to the fusion of two colored findings we can get the diagnosis more easily than by inspection of two different roentgenograms. This method applied for proper aims may have an advantage over the customary roentgenography, in that we can detect concealed foci, record the motion of a working organ, and inspect the otherwise flat image in its three dimensions.

One month after our publication there came out Dr. Donovans preliminary opinion on "Radiography in Color."[2] The technique he may adopt in the future seems to be what we call "Technicolor method."

We are indebted Dr. eng. Shinkichi Otani, Cheif of the Chigasaki Factory, Dai Nippon Toryo Co., Ltd. for his kind supplying various kinds of intensyfying screen for us.

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2) Donovan, G.E., Lancet., 1951, **260**, 832–833.

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Blood Picture of Nutritional Dystrophy in Children; Effect of Folic Acid and Vitamin B_{12} upon It

(Studies on the Nutrition of Children in Hirosaki Area, 10the Report)

By

Shigeho Wada and **Tsuneo Arakawa**
(和田 重穂) (荒川 雅男)

(From the Department of Pediatrics, Faculty of Medicine, Hirosaki University, Director: Prof. Ts. Arakawa)

(Received for publication, December 13, 1951)

As has already been reported by us[1], nutritional dystrophy[2] or infantile pellagra has been of endemic occurrence in Hirosaki Area in Tsugaru District, and macrocytic anemia is more frequently found in sick children with pellagrous or edematous form of nutritional dystrophy than in those with ariboflavinotic form.[1]

The sick children subjected to blood examination in the preceeding investigation[1] were those who had visited our Department with complaints of fever, coughing and etc., and shown to some extent clinical manifestations of nutritional dystrophy[2] on physical examination.

In the present paper we shall report the results of hematological investigation of a group of the school children from 7 to 12 years of age in Miyoshi village,[3] who showed, though apparently healthy, clinical manifestations of the pellagrous form of nutritional dystrophy to greater or smaller extent. The effect of folic acid respectively vitamin B_{12} upon the blood picture in these cases will also be reported.

Method and Materials

Blood examinations were made on peripheral blood from ear lobes, and for the differential count of white cells Sato's peroxidase stain[4] was used throughout this investigation. For reticulocyte count brilliant cresyl blue stain method,[5] for estimation of hematocrit values Kato's tubes were employed, and volume index was calculated from Andresen's table.[5] The present investigation was performed at Miyoshi village[3] in winter (from December 1950 to Feburary 1951) and in summer (May 1951).

TABLE I, A

Blood Pictures of Children with Nutritional Dystrophy during Summer (May 1951)

No.	Name, Sex	Age (Y.M.)	Ang.* stom.	Papillae Mush.*	Papillae Atr.*	Pig-ment.*	Hyper-kerat.*	R.C.* (m)	H.C.* (%)	V.I.*	H.b.* (g%)	C.I.*
1	M.M. ♀	6.2	(+)	(++)	(+)	(+)	(−)	4.59	45	1.08	12	0.87
2	K.T. ♀	6.3	(−)	(+)	(−)	(++)	(−)	3.52	42	1.00	13	0.49
3	K.M. ♀	6.11	(±)	(−)	(−)	(++)	(−)	4.13	41	1.09	11.5	0.93
4	N.H. ♀	6.11	(++)	(+)	(+)	(+++)	(++)	3.91	40	1.13	11	0.94
5	K.K. ♂	6.8	(+)	(−)	(+++)	(+++)	(−)	4.07	40	1.00	11.5	0.94
6	N.K. ♂	6.9	(++)	(+)	(++)	(+++)	(++)	4.13	37	1.01	12	0.97
7	N.H. ♂	6.3	(++)	(+)	(+)	(±)	(+)	4.11	41	1.10	12	0.98
8	W.M. ♀	6.11	(±)	(+)	(++)	(+)	(+)	3.86	40	1.10	11	0.95
9	S.S. ♀	6.5	(+)	(−)	(++)	(+)	(−)	4.52	39	0.95	11.5	0.85
10	Y.K. ♂	7.0	(+++)	(++)	(+++)	(+)	(++)	3.95	38	0.93	11	0.93
11	S.K. ♀	7.0	(±)	(−)	(+++)	(++)	(−)	4.76	37	0.85	11	0.82
12	T.K. ♂	7.11	(++)	(+)	(+)	(++)	(+)	4.13	39	1.04	12	0.97
13	M.K. ♀	8.8	(+)	(−)	(−)	(±)	(−)	4.11	40	1.06	10	0.79
14	N.S. ♂	8.1	(+++)	(−)	(+++)	(++)	(++)	4.51	40	0.96	12.5	0.88
15	O.A. ♀	8.8	(+)	(++)	(++)	(+)	(−)	4.19	41	1.06	12	0.93
16	N.Y. ♀	8.5	(++)	(+++)	(+)	(+)	(±)	4.27	38	0.97	11.5	0.87
17	K.T. ♂	8.9	(−)	(+)	(+++)	(+)	(+)	4.26	39	0.99	12	0.91
18	K.M. ♀	8.2	(−)	(+)	(−)	(+)	(±)	4.09	43	1.14	12	0.94
19	K.S. ♂	8.1	(+)	(++)	(++)	(−)	(−)	4.38	41	1.02	13	0.96
20	S.M. ♂	9.0	(±)	(−)	(+++)	(++)	(±)	3.89	38	1.06	11	0.91
21	K.K. ♂	9.1	(±)	(++)	(++)	(+)	(+)	4.22	41	1.05	12.5	0.95
22	K.K. ♂	9.0	(±)	(+)	(+)	(++)	(+)	4.11	37	0.98	12	0.94
23	K.C. ♂	9.0	(++)	(++)	(++)	(+)	(−)	3.54	35	1.08	11	1.00
24	S.S. ♀	9.1	(+)	(++)	(+++)	(+)	(+)	3.76	36	1.06	11	0.94
25	N.F. ♀	9.2	(++)	(++)	(+++)	(±)	(±)	4.39	36	0.91	11.5	0.78
26	O.C. ♀	9.5	(−)	(−)	(++)	(+)	(+)	4.69	44	1.01	13	0.89
27	K.I. ♀	9.9	(+)	(+)	(++)	(+)	(−)	4.38	46	1.14	14	1.03
28	N.K. ♂	9.10	(+)	(−)	(+)	(++)	(+)	3.09	34	1.19	12	1.35
29	K.S. ♀	9.10	(++)	(−)	(++)	(+++)	(+)	4.41	38	0.94	12.5	0.91
30	M.D. ♂	9.6	(++)	(−)	(+)	(++)	(++)	3.71	37	1.08	11	0.95
31	S.T. ♂	10.1	(+++)	(++)	(+)	(+)	(++)	4.50	43	1.03	12.5	0.89
32	K.N. ♂	10.1	(−)	(+)	(−)	(+)	(−)	4.10	39	1.03	12	0.94
33	S.N. ♂	10.4	(++)	(−)	(−)	(+)	(+)	4.22	39	1.00	14	1.06
34	T.Y. ♂	10.9	(+++)	(++)	(−)	(+++)	(++)	4.73	44	1.01	12.5	0.85
35	N.S. ♂	10.3	(+)	(−)	(−)	(++)	(−)	4.32	37	0.93	12	0.89
36	O.H. ♂	10.3	(−)	(−)	(+)	(++)	(++)	4.14	40	1.05	12	0.94
37	N.H. ♂	10.2	(+)	(−)	(++)	(±)	(−)	3.49	39	1.22	12	1.11
38	K.S. ♀	10.10	(±)	(−)	(+++)	(++)	(−)	4.20	40	1.04	12	0.92

Remarks: R.C.*=Red cells; H.C.*=Hematocrit; V.I.*=Volume index; H.b.*=Hemoglobin; C.I.*=Colour index; Ang. Stom.*=Angular Stomatitis; Mush.*=mushroom appearance; Atr.=Atrophy; Pigment.=Pigmentation; hyperkerat.*=Hyperkeratosis.

TABLE I, B

Blood Pictures of Children with Nutritional Dystrophy during Summer (May 1951)

No.	White count	Differential white count Absolute number and (%)				Nuclear shift of neutrophils (%)				
		Eosin.	Lymph.	Mono.	Neutro.	I	II	III	IV	V
1	11,800	413 (3.5)	6608(56)	531 (4.5)	4240(36)	4	32	48	3	3
2	9,400	3055(32.5)	2491(26.5)	188 (2)	3666(39)	16	37	38	9	0
3	10,400	312 (3)	3588(34.5)	520 (5)	5980(57.5)	6	35	43	13	3
4	3,400	306 (9)	952(28)	255 (7.5)	1887(55.5)	9	42	41	6	2
5	11,400	5110 (5)	2736(24)	912 (8)	7182(63)	11	36	46	7	0
6	9,000	540 (6)	2835(31.5)	270 (3)	5355(59.5)	7	48	37	8	0
7	11,800	236 (2)	4602(39)	1359(11.5)	5605(47.5)	5	33	43	17	2
8	9,200	1150(12.5)	4600(50)	506 (5.5)	2944(32)	12	46	29	8	5
9	7,000	140 (2)	4655(66.5)	140 (2)	2095(29.5)	4	32	44	18	2
10	9,000	495 (5.5)	3420 (3.5)	315 (3.5)	4770(53)	7	36	39	15	3
11	9,600	1248(13)	2928(30.5)	816 (8.5)	4608(48)	9	34	44	11	2
12	5,800	377 (6.5)	3248(56)	203 (3.5)	1972(34)	13	30	42	14	1
13	8,200	615 (7.5)	3977(48.5)	451 (5.5)	3157(38.5)	11	33	45	9	2
14	8,400	126 (1.5)	3612(43)	252 (3)	4788(57)	2	39	46	12	1
15	4,400	987(10.5)	3572(38)	282 (3)	5499(58.5)	11	34	44	10	1
16	8,000	280 (3.5)	5440(68)	360 (4.5)	1920(24)	6	33	47	14	0
17	7,200	1008(14)	1764(24.5)	396 (5.5)	4032(56)	14	36	30	16	4
18	9,600	384 (4)	4224(44)	288 (3)	4704(49)	17	33	37	13	0
19	7,000	245 (3.5)	2660(38)	315 (4.5)	3780(54)	13	51	33	3	0
20	5,200	308 (4)	2730(52.5)	338 (6.5)	1924(37)	17	37	36	5	0
21	5,200	390 (7.5)	2078(51.5)	182 (3.5)	1950(37.5)	14	30	42	14	0
22	9,200	322 (3.5)	2944(32)	966(10.5)	4968(54)	10	28	42	15	5
23	5,400	216 (4)	3105(57.5)	135 (2.5)	1944(36)	11	36	37	12	4
24	7,000	210 (3)	4340(62)	140 (2)	2310(33)	19	37	35	9	0
25	7,400	481 (6.5)	4233(54.5)	444 (6)	2442(33)	7	36	40	13	4
26	7,000	455 (6.5)	4059(58.5)	455 (6.5)	1995(28.5)	23	54	17	6	0
27	6,000	270 (4.5)	2550(42.5)	300 (5)	2880(48)	12	34	45	9	0
28	11,000	1870(17)	5500(50)	220 (2)	3410(31)	22	44	26	8	0
29	9,200	414 (4.5)	3404(37)	322 (3.5)	5060(55)	1	26	47	20	6
30	8,22	738 (9)	4100(50)	184 (2)	3198(39)	16	58	20	6	0
31	14,200	1066 (7.5)	4544(32)	284 (2)	8307(58.5)	14	52	30	4	0
32	6,000	390 (6.5)	1950(32.5)	3000 (5)	3360(50)	8	40	40	6	6
33	13,000	585 (4.5)	5070(39)	715 (5.5)	6630(51)	9	32	51	8	0
34	12,000	960 (8)	5100(42.5)	980 (6.5)	5160(43)	20	37	39	4	0
35	3,600	90 (2.5)	2142(59.5)	108 (3)	1260(35)	8	30	37	11	4
36	8,600	645 (7.5)	3139(36.5)	688 (8)	4128(48)	10	42	34	14	0
37	7,800	243 (3)	4134(53)	351 (4.5)	3081(39.5)	13	32	35	15	5
38	7,200	108 (1.5)	4536(63)	288 (4)	2268(31.5)	8	25	43	20	4

TABLE II, A

A. Blood Pictures of Children with Nutritional Dystrophy before and after Treatment with Folic acid during Winter

No.	Name, Sex	Age (Y.M.)	Reticulo. (%)	R.C.*	Mean of diameter (μ) of reds	H.C.* (%)	V.I.*	H.b.* (g%)	C.I.*	Date of experiment	Remarks
1	N.M. ♂	7.8		4.20	7.81	43	1.125	12.5	0.99	20/XII–1950	24, 25/XIII 1950 Folic acid 15 mgrms. respectively
				4.35		42	1.06	12.5	0.95	26/XII–1950	
2	O.K. ♂	7.8		3.74	8.27	42	1.23	11	0.98	21/XII–1950	23, 24 and 25/XII 1950 Folic acid 15 mgrms. respectively
				4.60		44	1.05	13	0.94	25/XII–1950	
3	O.K. ♂	7.8		3.90	8.12	44	1.24	11.5	0.98	21/XII–1950	23, 24 and 25/XII 1950 Folic acid 15 mgrms. respectively
				3.85	7.66	43	1.22	11.5	1.00	25/XII–1950	
4	K.N. ♂	8.5	12	3.46	6.84	38	1.19	10	0.93	29/I –1951	30 and 31/XII–1950 Folic acid 15 mgrms. respectiuely
			11	4.00	6.80	38	1.03	10	0.80	1/II –1951	
5	K.K. ♂	7.10	11	4.08	7.29	43	1.15	12.5	1.02	20/I –1951	30 and 31/1 1951 Folic acid 15 mgrms. respectively
			15	4.50	6.88	40	0.97	11	0.81	1/II –1951	
6	N.Y. ♂	8.3	10	3.70	8.61	46	1.34	11	0.96	26/I –1951	30 and 31/1 1951 Folic acid 15 mgrms. respectively
			23	4.70	7.09	46	1.06	12.5	0.82	1/II –1951	
7	S.O. ♀	8.10	13	3.92	7.215	42	1.16	11	0.90	26/I –1951	30 and 31/1–1951 Folic acid 15 mgrms. respectively
			12	4.65	6.90	39	0.91	11.5	0.71	1/II –1951	
8	N.S. ♂	8.10		4.25	7.485	44	1.12	11	0.85	26/I –1951	25, 26 and 27/XII–1950 Folic acid 15 mgrms. respectively
				4.50	6.95	43	1.03	13.5	0.97	2/II –1951	
9	M.T. ♂	9.7	6	3.60	7.68	46	1.39	12	1.07	23/XII–1950	30/I and 1/II–1951 Folic acid 15 mgrms. respectively
			9	4.36	7.445	46	1.14	12	0.88	30/XII–1950	
10	O.H. ♂	10.0	9	4.05	7.45	45	1.21	13	1.03	29/I –1951	30 and 31/1–1951 Folic acid 15 mgrms. respectively
			20	4.26	7.42	45	1.15	12	0.91	9/II –1951	
11	T.I. ♀	10.0	32	3.82	7.31	43	1.22	12	1.01	26/I –1951	30 and 31/1 1951 Folic acid 15 mgrms. respectively
			19	4.35	6.97	38	0.94	11	0.81	1/II –1951	
12	T.Y. ♂	10.4	17	4.30	7.445	44	1.12	13	0.98	26/I –1951	30 and 31/1–1951 Folic acid 15 mgrms. respectively
			17	4.47	7.485	47	1.14	13.5	0.97	2/II –1951	
13	A.K. ♂	10.9	10	4.00	8.03	44	1.19	10.5	0.84	27/I –1951	30 and 31/1– 1951 Folic acid 15 mgrms. respectively
			7	4.00	7.54	44	1.19	11.5	0.92	3/II –1951	
14	T.Y. ♂	11.0	14	3.30	7.6	40	1.30	9.5	0.92	27/I –1951	30 and 31/1–1951 Folic acid 15 mgrms. respectively
			28	4.35	6.77	36	0.87	10	0.74	2/II –1951	
15	N.I. ♂	12.9	17	4.10	7.405	42	1.11	11	0.86	15/II –1951	15 and 16/11–1951 Folic acid 15 mgrms. respectively
			21	4.40	7.46	43	1.05	12	0.88	19/II –1951	
16	I.S. ♀	12.8	19	3.90	7.46	44	1.22	13	1.07	15/II –1951	15 and 16/II–1951 Folic acid 15 mgrms. respectively
			18	4.55	7.38	44	1.06	13.5	0.95	19/II –1951	
17	K.T. ♂	10.9	6	3.55	7.51	42	1.3	13	1.19	14/II –1951	14 and 15/11–1951 Folic acid 15 mgrms. respectively
			18	4.45	7.1	43	1.04	10.5	1.04	19/II –1951	
81	K.F. ♂	11.6	17	3.95	7.3	41	1.11	91.5	0.78	14/II –1951	14 and 15/11–1951 Folic acid 15 mgrms. respectively
			12	4.30	7.15	40	1.00	12.5	0.93	19/II –1951	
19	T.Y. ♂	10.7	11	3.45	7.33	39	1.23	11	1.02	22/I –1951	30 and 31/1 1951 Folic acid 15 mgrms. respectively
			16	4.20	6.93	40	1.03	10	0.76	2/II –1951	

TABLE II, B

Blood Pictures of Children with Nutritional Dystrophy before and after Treatment with Folic acid during Winter

No.	White count	Differential white count Absolute number and (%)				Nuclear shift of neutrophils (%)				
		Eosinophils	Lymphocytes	Monocytes	Neutrophils	I	II	III	IV	V
1	11,600	580(5)	4060(35)	638 (5.5)	6322(54.5)	5.5	30.2	44	15.6	4.7
	12,500									
2	11,000	1540(14)	5390(49)	495 (4.5)	3575(32.5)	6	33	47	9	5
	8,200	984(12)	2624(32)	410 (5)	4182(15)	5	43	40	9	3
3	9,000	0 (0)	3150(35)	180 (2)	5670(63)	15	44	32	6	3
	7,500	75 (1)	2850(38)	450 (6)	4125(55)	2	33	54	9	2
4	10,000	1150(11.5)	4500(45)	350 (3.5)	4000(40)	5	38	45	10	2
	9,000	855 (9.5)	4410(49)	315 (3.5)	3420(38)	4	38	43	13	2
5	8,000	160 (2)	2520(31.5)	440 (5.5)	4880(61)	9	39	40	9	3
	7,600	380 (5)	3344(44)	380 (5)	3496(46)	6.5	43.5	41.3	6.5	2.2
6	6,200	93 (1.5)	3100(50)	372 (6)	2635(42.5)	1	28	45	21	0
	6,000	90 (1.5)	2340(39)	330 (5.5)	3240(54)	3	27	44	22	4
7	7,000	315 (4.5)	3115(44.5)	490 (7)	3080(44)	8	36	43	13	0
	6,000	300 (5)	2370(39.5)	330 (5.5)	3000(50)	19	42	32	7	0
8	8,400	336 (4)	3612(43)	588 (7)	3864(46)	2	33	48	13	4
	9,000	90 (1)	3150(35)	450 (5)	5310(59)	5	30	44	17	4
9	8,000	200 (2.5)	2880(36)	520 (6.5)	4400(55)	26	44	25	5	1
	7,400	259 (3.5)	3256(44)	333 (4.5)	3552(48)	21	36	39	4	0
10	16,200	960 (6)	8640(54)	320(20)	6080(38)	4	38	42	13	3
	12,000	960 (8)	5040(42)	780 (6.5)	5220(43.5)	7	41	42	7	3
11	4,500	247 (5.5)	1598(35.6)	293 (6.5)	2362(52.5)	5	36	42	17	0
	5,400	324 (6)	2511(46.5)	270 (5)	2295(42.5)	0	35	43	17	5
12	10,000	1800(18)	3700(37)	500 (5)	4000(40)	5	37.5	45	10	2.5
	6,200	558 (9)	2511(40.5)	372 (6)	2359(44.5)	21	36	33	10	0
13	5,000	400 (8)	2150(43)	350 (7)	2100(42)	7	29	45	14	5
	4,400	220 (5)	2728(62)	220 (5)	1232(28)	10	35	45	10	0
14	8,200	820(10)	3116(38)	246 (3)	4018(49)	1	30	41	22	6
	4,000	540(13.5)	1560(39)	220 (5.5)	1680(42)	7.2	23.8	56	13	0
15	6,000	210 (3.5)	2430(40.5)	300 (5)	3060(51)	26	42	28	4	0
	7,000	315 (4.5)	3290(47)	385 (5.5)	3010(43)	33	54	13	0	0
16	9,200	874 (9.5)	2852(31)	598 (6.5)	4875(53)	2	35	43	17	3
	8,000	440 (5.5)	2560(32)	240 (3)	4760(49.5)	9	39	45	7	0
17	5,600	280 (5)	2576(46)	252 (4.5)	2492(44.5)	2	41	47	10	0
	6,000	120 (2)	2220(37)	360 (6)	3300(55)	20	45	29	66	0
18	8,600	774 (9)	5031(58.5)	387 (4.5)	2408(28)	2	34	44	20	0
	9,600	960 (10)	4032(42)	384 (4)	4224(44)	8	37	43	10	2
19	12,000	480 (4)	3600(30)	600 (5)	7320(61)	5	44	45	6	0
	9,200	46 (0.5)	2622(28.5)	322 (3.5)	6310(67.5)	10	35	46	10	0

TABLE

Blood Pictures of Children with
after Treatment with

No. of case	Name and sex	Age (y. m.)	Red (millions)	Reticulocytes (‰)	Distribution (%) of diameter (μ) of reds							Hematocrit (%)	Volume index	Hemoglobin (g %)	Color index	Total white cells	Eosinophils
					5	6	7	8	9	10	11						
1	I.M.	7.1	3.73		0	6	47	36	10	1	0	40	1.17	9.5	0.84	17,000	425
	♂		3.77									39	1.13	11	0.91	8,600	
2	K.K.	6.10	3.76		0	2	44.5	43	9	1.5	0	40	1.18	11.5	1.02	10,000	150(1.5)
	♂		4.00		0	5	50	38	7	0	0	40	1.09	11	0.91	10,000	150(1.5)
3	K.K.	8.7	3.76		0	3	42	37	17	1	0	41	1.18	12	1.03	6,600	264(4)
	♀		3.95		0	7	50	38	6	0	0	42	1.15	13	1.05	8,000	520(6.5)
4	K.Ch.	8.7	3.94		1	12	53	30	3	1	0	40	1.10	11	0.90	6,800	0(0)
	♀		4.38									40	0.99	11	0.81	7,000	
5	K.M.	8.7	3.75	14	0.5	9	61	28.5	1	0	0	40	1.15	9	0.77	8,000	40(0.5)
	♂		3.92	15	0	6	57.5	34	2.5	0	0	43	1.19	10	0.82	8,800	440(5)
6	T.K.	9.6	3.92	8	0	6	63	30	3	0	0	40	1.11	11	0.90	6,400	224(3.5)
	♂		4.15	7	0.5	3	56.5	34	6	0	0	44	1.15	11	0.85	5,000	225(4.5)
7	K.B.	9.5	3.67	7	0	5	69	24	2	0	0	42	1.24	10.5	0.92	10,000	100(1)
	♂		4.00	17	0	9	65	25	1	0	0	46	1.25	12	0.96	10,000	300(3)
8	K.T.	9.1	3.70	10	0	2	55	40	3	0	0	40	1.17	10	0.84	8,000	960(12)
	♀		4.80	8	0	3	64	27	4	0	0	42	0.95	10	0.69	5,000	350(7)
9	N.O.	9.3	3.85	7	0	4.5	52	37.5	5.5	0	0	44	1.24	12	1.00	6,600	330(5)
	♀		3.90	10	0	7.5	50	37	5.5	0	0	46	1.28	12	0.99	6,600	759(11.5)
10	N.S.	9.0	3.57	5	0	11.5	50	34.5	13.0	1	0	38	1.16	10	0.90	6,000	150(2.5)
	♀		4.05	8	0	10.5	53	33	3.5	0	0	42	1.13	10.5	0.83	4,000	40(1)
11	W.K.	9.3	3.70	7	0	7.5	52	30	7.5	3.5	0	40	1.17	9.5	0.82	6,000	60(1)
	♂		4.50	9	2	14	60	21	3	0	0	42	1.01	10	0.86	7,000	100(1.5)
12	N.S.	9.7	3.80	10	3.5	19	51.5	24.5	1.5	0	0	42	1.20	9.5	0.80	6,000	450(7.5)
	♂		4.20	10	3.5	14.5	58.5	3.5	1.0	0	0	40	1.03	10	0.76	8,400	630(7.5)
13	S.M.	10.1	3.40	7	0	1	36	46	15	2	0	43	1.37	10	0.94	6,000	750(12.5)
	♀		3.85	8	0	3	52.5	38.5	6	0	0	41	1.15	12.5	1.04	7,400	296(4)
14	N.E.	10.5	3.95	9	1	7	51	37	4	0	0	43	1.18	10	0.81	7,000	350(5)
	♀		4.40	9	0	4.5	58.5	30	7	0	0	41	1.31	10.5	0.99	7,600	380(5)

III

Nutritional Dystrophy before and Vitamin B_{12} during Winter

Differential count of white cells absolute number and (%)			Nuclear shift of neutrophils (%)					Day of experiment	Remarks
Lymohocytes	Monocytes	Neutrophils	I	II	III	IV	V		
4080	340	12155	1.4	16.7	40.5	32.1	9.3	20/XII 1950	22, 24 and 25/VIII 1950 B_{12} 15γ each day
4900(49)	650 (6.5)	4300(43)	16.2	41.8	36	6	0	21/VII 1950	23, 24 and 25/XII 1950
6300(63)	250 (2.5)	3300(33)	15	39	42	4	1	26/XII 1950	B_{12} 15γ each day
2504(38)	594 (9)	3234(49)	4	26	44	24	2	23/VII 1950	25, 26 and 26/XII 1950
3280(41)	440 (5.5)	3760(47)	4	32	48	13	3	30/XII 1950	B_{12} 15γ each day
1224(18)	476 (7)	5100(75)	23	48	25	3	1	23/XII 1950 28/XII 1950	25, 26 and 20/XII 1950 B_{12} 15γ each day
2520(31.5)	960 (12)	4480(56)	8	42	44	4	2	26/I 1950	31/I and 3/II 1951
4928(56)	682 (3)	2510(31)	11	48	36	5	0	12/II 1951	B_{12} 12γ each day
2496(39)	320 (5)	3360(52.5)	5	33	42	16	4	26/II 1951	31/I and 3/II 1951
1725(34.5)	250 (5)	2700(56)	3	42	46	9	0	13/II 1951	B_{12} 15γ each day
4350(43.5)	500 (5)	5050(50.5)	9	33	46	9	3	29/I 1951	31/I and 3/II 1951
5400(54)	500 (5)	3800(38)	4	23	52	17	4	13/II 1951	B_{12} 15γ each day
4240(53)	240 (3)	2560(32)	6	34	47	13	0	30/I 1951	30/I and 3/II 1951
2750(55)	180 (3)	1750(35)	4	34	48	11	3	13/II 1951	B_{12} 15γ each day
3033(50.5)	561 (8.5)	2376(36)	16	47	35	2	0	30/I 1951	31/I and 3/II 1951
3663(55.5)	528 (8)	1650(25)	12	45	42	1	0	13/II 1951	B_{12} 15γ each day
2850(47.5)	300 (5)	2700(45)	17	44	33	6	0	29/I 1951	30/I and 3/II 1951
1600(49)	240 (6)	2120(53)	37	41	19	3	0	13/II 1951	B_{12} 15γ each day
2610(43.5)	150 (2.5)	3180(53)	5	34	47	11	3	31/I 1951	31/I and 5/II 1951
2835(40.5)	280 (4)	3780(54)	3	37	46	11	3	14/II 1951	B_{12} 15γ each day
2580(43)	210 (3.5)	2760(46)	9	35	v43	9	4	31/I 1951	31/I and 5/II 1951
2640(31.5)	336 (4)	7488(57)	5	33	44	14	4	14/II 1951	B_{12} 15γ each day
1500(26.5)	180 (3)	3480(53)	10	48	36	6	0	31/I 1951	1/II and 5/II 1951
2442(33)	512 (7)	4144(56)	3	37	46	11	3	14/II 1951	B_{12} 15γ each day
3255(46.5)	420 (6)	2975(42.5)	16	40	30	5	0	26/I 1951	31/I and 3/II 1951
3214(41.5)	796 (10.5)	3268(43)	18	51	25	6	0	12/II 1951	B_{12} 15γ each day

TABLE IV

Blood Pictures of Children with Nutritional Dystrophy during Winter

No. of case	Name and sex	Age (y. m.)	Angular stomatitis	Mushroom like appearance	Atrophy	Pigmentation	Hyperkeratosis	Liver palpable Left mammillary line	Edema	Reds (millions)	Hematocrit (%)	Volume index	Hemoglobin (g%)	Color index	Total white cells	Differential count of white cellls absolute number and (%) Eosino-phils	Lympho-cytes	Mono-cytes	Neutro-phils	Nuclear shift of neutrophils (%) I	II	III	IV	V	Date of experiment
1	K.F. ♂	7.4	(+)	(++)	(−)	(+)	(+++)	(+)	(±)	4.20	41	1.07	10.5	0.83	9,600	528 (5.5)	4656 (48.5)	576 (6)	3840 (40)	10	45	40	5	0	21/XII 1950
2	N.A. ♂	7.6	(−)	(+)	(−)	(+)	(+++)	(−)	(−)	4.00	40	1.09	10.5	0.87	6,000	300 (5)	1920 (32)	240 (4)	3540 (59)	7	38	38	15	2	21/XII 1950
3	N.K. ♂	8.10	(−)	(−)	(+)	(++)	(+++)	(−)	(+)	4.10	36	0.95	11	0.79	9,000	450 (5)	3690 (41)	540 (6)	4320 (48)	6	35	46	8	0	30/I 1951
4	K.T. ♂	8.10	(−)	(+)	(−)	(+)	(++)	(−)	(−)	4.37	43	1.06	11	0.81	4,000	280 (7)	1920 (48)	160 (4)	1640 (41)	12	48	29	7	4	30/I 1951
5	O.K. ♂	8.10	(±)	(+)	(−)	(+)	(+++)	(−)	(−)	4.04	40	1.07	10.5	0.83	6,000	0 (0)	4140 (69)	120 (2)	1740 (29)	34	41	25	0	0	29/I 1951
6	N.M. ♂	8.11	(+)	(−)	(+)	(++)	(+++)	(−)	(−)	3.00	43	1.19	12	0.99	6,000	360 (6)	3180 (53)	120 (2)	2340 (39)	5	23	48	20	4	31/I 1951
7	K.T. ♀	8.11	(+)	(++)	(−)	(+)	(++++)	(−)	(−)	3.60	42	1.26	11.5	1.03	15,000	300 (2)	4050 (27)	300 (2)	10360 (69)	23	46	23	8	0	31/I 1951
8	K.Y. ♀	9.0	(+)	(++)	(−)	(+)	(+++)	(−)	(−)	4.20	38	0.98	10.5	0.8	6,000	240 (4)	1920 (32)	240 (4)	3600 (60)	8	40	40	12	0	31/I 1951
9	T.A. ♀	9.0	(−)	(−)	(−)	(±)	(+++)	(−)	(−)	3.55	32	0.98	9	0.81	9,000	810 (9)	5400 (60)	360 (4)	2430 (27)	7	30	41	19	3	23/XII 1950
10	S.Y. ♀	11.7	(−)	(++)	(−)	(++)	(+++)	(−)	(−)	4.65	42	0.98	10	0.68	8,000	0 (0)	2960 (37)	400 (5)	4640 (58)	22	51	25	2	0	14/II 1951
11	K.I. ♂	11.9	(+)	(−)	(−)	(+++)	(+++)	(−)	(−)	4.1	39	1.05	11	0.86	7,000	280 (4)	1400 (20)	350 (5)	4970 (71)	18	42	33	7	1	14/II 1951
12	K.K. ♀	12.10	(−)	(−)	(−)	(++)	(++)	3f.b.(+)		4.45	44	1.06	11.5	0.83	4,400	374 (8.5)	1474 (33.5)	220 (5)	2332 (53)	5	36	44	12	1	15/II 1951
13	K.Y. ♀	12.6	(±)	(+)	(−)	(++)	(++)	(−)	(−)	4.15	42	1.09	11.5	0.89	5,200	728 (14)	2158 (41.5)	494 (9.5)	1820 (35)	11	47	36	6	0	15/II 1951

Results

1. Blood pictures of children with nutritional dystrophy in winter and in summer (Cf. Tables I, II, III and IV).

Blood pictures obtained during the winter season are given in Tables II, III and IV and those obtained in summer in Tables I. Macrocytic anemia was found in 35 cases (76.0%) out of the 46 cases examined in winter, and in only 5 cases (13.1%) out of the 38 cases examined in summer. The incidence of macrocytic anemia in the children with nutritional dystrophy is higher with statistically significant difference in winter than in summer. Out of the 46 cases examined in winter neutropenia was found in 16 cases and eosinophilia in 27 cases. Out of the 38 cases examined in summer neutropenia was found in 14 cases and eosinophilia in 19 cases. Respecting incidence of neutropenia or eosinophilia, there was no seasonal difference of any statistical significance between summer and winter.

2. Effect of folic acid upon blood pictures (Cf. Table II).

Folic acid,* was injected subcutaneously, in an amount of 15 mgrms. per day for 2 or 3 successive days, into 19 cases from among the 40 cases with macrocytic anemia. As will be seen from Table III, the effect of folic acid upon blood pictures was summarized as follows: reds were increased in 17 cases (Cases 1, 2, 4~6, 7~12 and 14~19); reticulocyte response was found in 8 cases (Cases 5, 6, 9, 10, 14, 15, 17 and 19) out of 15 cases examined; volume index was returned to normal in 17 cases (Cases 1~10, 11, 14~19); total count of white cells was decreased in 14 cases (Cases 2~10, 12~14, 16 and 18~19); eosinophile count decreased in 12 cases (Cases 2~4, 6~8, 10, 12, 14, 16 and 18~19) and color index was reduced slightly in a majority of the cases.

3. Effect of vitamin B_{12} on blood pictures (Cf. Table III).

Subcutaneous injection of vitamin B_{12}† was carried out in 14 cases from among the 40 cases with macrocytic anemia in an amount of 15 γ per day for 2 or 3 consecutive days. The effect of vitamin B_{12} on blood pictures was summarized as follows; red cell count was elevated in 12 cases (Cases 2~12 and 13); reticulocyte response was found only in one case (Case 7) out of 11 cases examined; volume index was returned to the normal in 7 cases (Cases 2, 3, 4, 8, 11, 12 and 13) and remained unchanged in the other 6 cases; respecting color index no remarkable change

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* "Foliamin" is a proprietary name for folic acid (Takeda Pharmaceutical Industries).

† "Fresmin" is a proprietary name for vitamin B_{12} (Takeda Pharmaceutical Industries).

were found; total count of white cells was increaxsed in 7 cases (Cases 3, 4, 5, 11, 12, 13 and 14), decreased in 4 cases (Cases 1, 6, 8 and 10) and showed no changes in 3 cases (Cases 2, 7 and 9); eosinophile count was decreased in 3 cases (Cases 8, 10 and 13), increased in 7 cases (Cases 3, 5, 7, 9, 11, 12 and 14) and showed no changes in 3 case (Cases 2, 4, and 6).

Discussion

Spies et al.[6-7] reported effectiveness of folic acid upon nutritional macrocytic anemia and in 1949; they reported successful treatment with vitamin B_{12} in 17 cases of nutritional macrocytic anemia. In 1949 Woodruff et al.[8] described that out of 5 infants of macrocytic anemia 3 cases responded to folic acid and the other two to vitamin B_{12} respectivelly. Respecting pathogenesis of macrocytic anemia in infancy, May et al[8] stated that, for a primary cause, chronic vitamin C deficiency would be responsible by which secondarily metabolism of folic acid might be disturbed. According to Israls and Sharp,[10] macrocytic anemia may be devided into 2 groups: the first includes pernicious anemia and nutritional anemia found in Southern U.S.A. and in the West India, and responds to vitamin B_{12} as well as folic acid; the second includes pernicious anemia of pregnancy, macrocytic anemia of idiopathic steatorrhea or tropical sprue, and scarcely responds to vitamin B_{12}, but does sharply to folic acid. Respecting macrocytic anemia of our own infantile cases with nutritional dystrophy at Miyoshi village in Tsugaru District, we may conclude, basing upon our investigation, that incidence of macrocytic anemia was higher in winter than in summer and that a decrease in volume index was obtained more markedly by folic acid therapy than by vitamin B_{12} treatment.

Etiological factor to such macrocytic anemia as seen among the children in our District still remains a question to us, but dietary deficiency especially in the winter season may probably play an important role. Neutropenia or eosinophilia was, as already described, found in a considerable number of the cases both in winter and in summer, but regarding incidence of neutropenia or eosinophilia there was, as above mentioned, no statistically significant difference of it between summer and winter. Decrease in total white cell count or in eosinophile count was observed more frequently in the cases treated with folic acid than those treated with vitamin B_{12}.

Summary and Conclusions

1. In summer and winter 1950–1951, blood picture was examined

on school children at a rural village (Miyoshi village) inTsguaru District. Though they were apparently healthy, yet they were found to be in a state of nutritional dystrophy on close examination.

2. Macrocytic anemia was found in 35 cases (76.0%) out of the 46 cases examined in winter, and in only 5 cases (13.1%) out of the 38 cases examined in summer.

3. Macrocytic anemia observed in winter responded sharply to folic acid and scarcely to vitamin B_{12}. The method of administration was subcutaneous.

4. Decrease in total white cell count or in eosinophile count was observed in a majority of the cases treated with folic acid, but only in a minority of those treated with vitamin B_{12}.

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Hepatomegalic Liver in Children with Chronic Miliary Tuberculosis and Liver Biopsy Findings

(Studies upon the Nutrition of Children in Hirosaki Area. 11th Report)

By

Yuji Sato and **Tsuneo Arakawa**

(佐藤 雄治) (荒川 雅男)

(From the Department of Pediatrics, Faculty of Medicine, Hirosaki University, Director: Prof. Ts. Arakawa)

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In Hirosaki Area, as already are reported by us[9] infantile pellagra or nutritional dystrophy has been of endemic occurrence. Now, an enenlargement of the liver is, as is well known, a very common finding in chronic miliary tuberculosis. But in this Area, not an enlarged liver, but a " hepatomegalic " liver will be found as a prominent feature among clinical manifestations of chronic miliary tuberculosis in children. We desire to report in the present paper the pathogenesis of the hepatomegaly in question on the basis of liver biopsy findings.

Report of Cases

Case 1. O.T., a girl aged 2 years and 11 months, was admitted into our Clinic on Sept. 25, 1950, with complaints of fever, cough and anorexia which had been persisting after an attack of ' pneumonia ' about a month prior to her entry.

She was poorly nourished and had a tongue with mushroom-like appearing papillae. Tuberculin test (1:1000) was positive. Snow falling picture was found on the roentgenogram of the lungs. Nothing abnormal could be detected over the lungs and the heart on physical examination. Cervical lymph nodes were enlarged moderately on both sides. The liver was palapable at four fingers' breadth below the costal margin on mid-clavicular line and the spleen was palpable at one and a half fingers' breadth below the costal margin. From Sept. 1950 to Jan. 1951 Streptomycin was given intramuscularly in an amount of 0.5 grms. per day. Towards the end of a 120 days long continued streptomycin treatment anorexia was gradually improved, but the liver remained unchanged in its size.

From March 31 to May 14, 1950, one grm. of methionine was given perorally every day. On the 8th day (April 7) of this peroral administration the liver had become smaller in its size, though it was still palpable at three fingers' breadth below the costal margin on mid-clavicular line, but thereafter the liver was gradually enlarged again, regaining its size prior to the administration of methionine. Liver biopsy was carried out on June 21, 1951.

Case 2. M.T., a girl of 6 1/2 years was admitted to our Clinic on April 6, 1951. For about a month prior to her entry, high fever, loss of appetite and prostration were complained of. On admission, she was a pale, poorly nourished girl with angular stomatitis and magenta tongue. There were no heart murmurs. Consonating rales were audible on both sides of the lungs and miliary tubercles of the lungs were detected by way of roentgenogram. The liver was palpable at three and a half fingers' breadth below the costal margin on mid-clavicular line and the spleen at one and a half fingers' breadth on mid-axillary line. Enlargement of superficial lymph nodes was not found.

Streptomycin was given intramuscularly in an amount of 0.5 grms. per day consecutively from April 8 to April 28, 1951. In addition to still continuing high fever, anorexia and emaciation, the absdomen became tender and began to distend gradually with an accumulation of fluid in the abdominal cavity.

Liver biopsy was carried out on June 21, 1951.

Case 3. M.Y., a girl aged 3 years and 8 months visited our Clinic on March 2, 1950, with complaints of remittent high fever, pallor and loss of appetite. On examination she was a poorly nourished girl with angular stomatitis and mushroom-like appearance of lingual papillae. The liver was palpable at four fingers' breadth below the costal margin on mid-clavicular line and the spleen at two fingers' breadth on mid-axillary line. Nothing abnormal was found over the heart and the lungs on physical examination. The roentgenogram, taken on March 2, 1950, showed widening of upper mediastinum and miliary tuberculosis of the lungs. The superficial lymph nodes were not palpable. Streptomycin was given intramuscularly, total dose amounting to 20 grms. during the period from March 2 to April 16, 1950, and Tebezon* was given orally from July 15 to December 20, 1950.

From April on, gradual improvement in general condition was achieved and her appetite increased steadily.

At the end of the year 1950, the liver was still palpable at three and a half fingers' breadth below the costal margin, and miliary shadows scattered through the lungs had decreased in both number and density.

Discussion

I. Liver biopsy findings

Ante-mortem histologic studies of hepatic tissue obtained by needle biopsy have, it seems, been carried out only in recent years. Here a brief bioptic description of our own cases will be made. In the 2 cases (Cases 1 and 2) biopsied by us, the method—the mid-clavicular line, subcostal approach—was employed, using a Mitsuki needle.[1] Fatty metamorphosis of the liver was found in both cases, and was more advanced in its grade in Case 2 than in Case 1: the latter had, as stated already in Report of Cases, been treated with methionine as well as with streptomycin. In addition to these findings, portal fibrosis was found in both cases, while miliary tubercles (of the liver) were identified in Case 2 only.

Janbon, Cazal and Bertrand[2] reported 45 liver biopsy findings in 24 patients with miliary tuberculosis, in which hyalinization and scarring of hepatic lesions were, as they stated, found following the treatment with streptomycin.

In 1951 Seife et al.[3] reported histologic investigations by needle biopsy of the liver in 70 cases of chronic active pulmonary tuberculsis and stated that fatty metamorphosis of the liver in tuberculous cases was not related to tuberculosis per se. They presumed that it might be ascribed to dietary deficiency.

II. Marked enlargement of the liver as a prominent feature among clinical manifestations of chronic miliary tuberculosis in children

In 1928 Greengard[4] reported that the spleen was palpable in 15 cases and the liver in 11 out of 54 cases of miliary tuberculosis of children. In 1935 Lincoln[5] stated that enlargement of the spleen and sometimes that of the liver were observed in cases with protracted hematogenous dissemination of children. In 1940 Simon[6] described that hepatosplenomegaly was frequently met with in acute forms, but not commonly in protracted form of miliary tuberculosis in children.

In 1939 Moridera[7] made clinical observations of 47 cases with miliary tuberculosis of children and described enlargement of the spleen in 34% out of all his cases, while respecting that of the liver he gave no description at all.

In 1950 Arakawa,[8] one of us, experienced 8 cases of miliary tuberculosis in children at the Tohoku University Hospital in Sendai and reported that in one case out of 3 cases of chronic form the liver was felt

at two fingers' breadth under the costal margin and that, in 3 cases out of 5 cases of acute form of miliary tuberculosis of children, the liver was felt in the first case at two fingers' breadth under the costal margin, in the second, at one finger's breadth, in the third at two fingers' breadth. It seems that in the hitherto literature, foreign as well as native, a hepatomegalic enlargement of the liver has not been a prominent feature among clinical manifestations of miliary tuberculosis in children. Indeed, chronic tuberculous affection is, as in well known, a consuming disease and, though it is indeed a predominant factor to make general nutrition of the victim much worse, yet as to the hepatomegalic liver in our own cases, a main cause must be sought in the nutritional dystrophy prevalent among children in this Area.

Liver biopsy findings in our own cases revealed, as above metnioned, portal fibrosis and fatty metamorphosis in Case 1 and miliary tubercles, periportal fibrosis and fatty infiltration to a greater extent in Case 2. And we want to state that in the former (Case 1) a reduction of enlargement of the liver—though even slight—was due to an administration of methionine. And the fact that the marked enlargment of liver very common among children with chronic miliary tuberculosis in Hirosaki Area will not, as just mentioned, be well explained by the assumption that the liver here is much more affected in general tuberculous affection. But the main cause is due to the liver affection due to the kind of nutritional dystrophy prevalent in this Area, and this affection is diffuse fatty metamorphosis of the liver, as we have shown on the basis of liver biopsy in the present investigation.

Summary

1. In Hirosaki Area infantile pellagra or nutritional dystrophy has been prevalent.

2. Marked enlargement of the liver as a prominent feature among clinical manifestations of chronic miliary tuberculosis in children in this Area should be regarded in a new light.

3. Liver biopsy was carried out in 2 cases of chronic miliary tuberculosis, and it revealed miliary tubercles, portal fibrosis and marked fatty infiltration of the organ.

4. Marked enlargement of the liver in these tuberculous children will be ascribed to fatty infiltration of the liver (identified by liver biopsy) probably due to dietary deficiency prior to their tuberculous infection.

Conclusions

The marked liver enlargement or hepatomegalic liver in children

of chronic miliary tuberculosis in Hirosaki Area is, taking liver biopsy findings into consideration, chiefly ascribed to the form of diffuse fatty liver due to nutritional dystrophy prevalent in the Area.

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* Tebezon is a proprietary name (Marupi Seiyaku Company) for para-acetylamino- benzaldehyde thiosemicarbazone.

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An Experimental Research of Bilateral Open Chest

I. The E.C.G. Findings during Bilateral Open Chest in Rabbits

By

Yoshinobu Ishikawa
(石川義信)

(From Prof. Katsura's Surgical Clinic, Faculty of Medicine, Tohoku University, Sendai)

(Received for publication December 14, 1951)

During the operation for cancer of upper esophagus in thorax, I was obliged to open both pleural cavities in 22% of 18 cases, as it has appeared in the literatures.[1)2)] In order to continue the operation in such cases, the respiration by positive pressure is absolutely necessary. Many researches concerning unilateral open chest have been reported, but not so many about bilateral open chest.

The following experiments were carried out to study the effects upon the heart exercised by the bilateral open chest under respiration by positive pressure.

As the studies concerning the influences of positive pressure during bilateral open chest upon E.C.G. have not yet been reported in the literature, I attempted to clarify some influences of various degree of positive pressure during open chest upon heart function by E.C.G.

Method

Rabbits weighing over 2 Kg., anesthetized by subcutaneous injection of 25% urethan 4.0 c.c. per Kg. were fixed in supine position. An incision was made from the posterior axillar line to the sternal margin in 6th intercostal space on both sides of the chest wall.

Oxygen was sent at the rate of 2 L. per minute through semiclosed apparatus into the trachea. The constant positive pressure of oxygen, 4 cm., 8 cm., and 16 cm. of water respectively, was maintained during the experiments. They were grouped into three, and in each group, the E.C.G. was led from the limbs at 10 minutes, 30 minutes, 1 hour and 2 hours after both chests had been opened and before operation, respectively. E.C.G. was amplified by a C-R coupled three staged amplifier and recorded photographically by an oscillograph (H. type vibrator, made

by Yokogawa Co.). The heights of the lowest and the highest waves fo E.C.G. pattern were estimated and the average of them was taken. Mean electrical axis of QRS was calculated by Jackson's method[3] and the intervals of each wave were also measured. The calculation of PQ-, QT-intervals were made by Shimizu- Fukuda's equation[4]: $PQ=2.32\sqrt[3]{RR}\pm0.025$ and $QT=2.83\sqrt{RR}\pm0.025$. Cases which showed an overlap of T and P were excluded.

Results

(1) Under anesthesia and supine fixation, isoelectric or inverted T_2 and T_3 could not recognized and the intervals of each wave were within the limits of normal reported by Shimizu-Fukuda.[4]

(2) In cases of 4 cm. of water constant positive pressure breathing (see Fig. 1 and 2, Table I): In one of 5 cases just after the open chest there appeared idioventricular rhythm, which, however, disappeared in a short tome.

RR interval increased above the normal limits in all cases except No. 3 and No. 17 after 10 minutes. *PQ interval:* Increase above the normal limits were found in No. 17 and No. 18 after 30 minutes. None shows the measured value lying outside the range predicted by Shimizu-Fukuda's equation. *QT interval:* The prolongation of *QT* was observed in all cases, but usually remained still within the normal value. Those which prolong above the normal limits were found in No. 17 after 30 minutes, in No. 19 after 30 minutes and 1 hour and in No. 18 after 30 minutes, respectively. The prolongation outside the range indicated by the equation were found in No. 18 after 30 minutes and 1 hour. *Mean QRS electrical axis:* The changes in the electrical axis by respiration became greater under open chest. The average of inspiratory and expiratory electrical axis, however, exhibited only slight changes (10° or so) compared with the preoperative value except in No. 19, No. 6 after 10 minutes, 30 minutes, respectively. None of these changes, however, were not so great as to be regarded distinct changes of type of the electrical axis deviation. *Pulmonary P* was regognized in No. 17 after 10 minutes. *T wave:* Each case (except T_1 of No. 6 after 10 minutes) shows a flat T, it is especially remarkable in T_3.

(3) In case of constant positive pressure of 8 cm of water (see Fig. 3, Table II.), *RR Interval:* It shortened in all cases except No. 7 after 30 minutes, No. 9 after 30 minutes, No. 10 after an hour, 2 hours, respectively, compared with the preoperative condition, but all were within the normal limits. *PQ interval* became short but was within the normal limits in all cases. No cases were found where the measured value was

TABLE I

ECG. of Bilateral Open Chest Under 4 cm. of Water Constant Positive Pressure

No. of case	Height of wave and time interval of waves / Course of time	P (m.m.) I	P (m.m.) II	P (m.m.) III	Q I	Q III	R I	R III	S I	S III	T I	T II	T III	P (sec)	P—Q Observed value	P—Q Calculated value	Q—T Observed value	Q—T Calculated value	T—P	R—R	Mean QRS electric axis
No. 6	Before op.	1.9	2.0	1.0	0	0	4.0	7.0	3.0	3·5	2.1	2.0	1.0	0.036	0.063	0.063	0.138	0.127	0.006	0.200	+77°
	10 min. after open chest	1.3	2.0	1.0	0	0	4.5	2.5	3.5	0.9	2.2	1.5	0	0.032	0.065	0.066	0.144	0.136	0.018	0.229	+68
	30 min. " "		2.0									2.0		0.015	0.079	0.068	0.153	0.141	0.015	0.248	
	1 hour " "	1.5	2.0	1.2	0	0	·3.3	3.5	1.4	2.0	2.0	2.0	0	0.041	0.062	0.062	0.125	0.125	0.006	0.203	+68
	2 hours " "	1.5	1.9	1.8	0.5	0	3.5	6.4	1.2	3.0	2.0	0	0	0.029	0.069	0.063	0.125	0.129	0.034	0·208	+70
No. 17	Before op.	0	2.1	1.0	1.9	0	5.1	6.7	0	1.5	1.5	2.0	2.1	0·046	0.083	0.067	0.128	0.139	0.131	0.347	+65°
	10 min. after open chest	0	2.1	2.8	2.0	0	6.2	3.4	0	0	1.0	1.0	0	0.044	0.087	0.072	0.147	0.153	0.059	0.296	+58
	30 min. " "	0	2.1	1.9	1.0	0	4.4	6.3	0	1.0	1.0	1.5	0	0.038	0.088	0.077	0.189	0.175	0.101	0.379	+67
	1 hour " "	0	2.0	1.7	1.0	0	5.1	3.0	0	0	0.5	1.0	0	0.039	0.092	0.079	0.171	0.178	0.131	0.396	+55
	2 hours " "	0	1.5	1.7	2.0	0	6.0	8.0	0	5.0	0	±	0.5	0.035	0.088	0.082	0.171	0.188	0.185	0.442	+55
No. 18	Before op.	1.0	2.4	2.0	0	0	0.5	5.5	0	1.0	1.8	2.1	1.5	0.028	0.071	0.065	0.115	0.132	0.034	0.220	+85°
	10 min. after open chest	1.0	1.9	1.3	0	0	0.8	6.0	0	1.4	1.3	1.0	0	0.029	0.080	0.071	0.131	0.142	0.005	0.271	+84
	30 min. " "	1.0	1.8	2.0	0	0	0.3	5.0	0	1.6	0.3	1.0	0	0.027	0.093	0.077	0.202	0.172	0.120	0.371	+87
	1 hour " "	0	1.1	0	0	0	0.3	4.5	0	0.5	0.3	1.0	0	0.025	0.073	0.075	0.179	0.163	0.130	0.332	+84
	2 hours " "	0	1.7	0	0	0	1.0	5.8	0	0.2	0	1.0	0	0.027	0.077	0.075	0.176	0.164	0.085	0.334	+82
No. 19	Before op.	1.0	2.8	0	0	0	3.0	8.0	0	3.0	1.0	3.3	2.0	0.031	0.062	0.069	0.156	0.145	0.037	0.262	+68°
	10 min. after open chest	1.0	1.7	0	0	0	0.6	5.0	0	0	1.0	1.0	0	0.027	0.070	0.072	0.163	0.155	0.067	0.298	+57
	30 min. " "	1.0	1.7	0	0	0	4.5	2.5	0	0	1.9	1.7	0	0.035	0.075	0.080	0.206	0.179	0.126	0.406	+51
	1 hour " "		1.5	0		0		8.0		0		2.8	0	0.023	0.069	0.097	0.236	0.232	0.432	0.736	
	2 hours " "	0.8	2.0	0	0	0	7.0	6.0	0	1.9	0.8	2.0	0	0.037	0.067	0.069	0.142	0.144	0.039	0.262	+54

Fig. 1

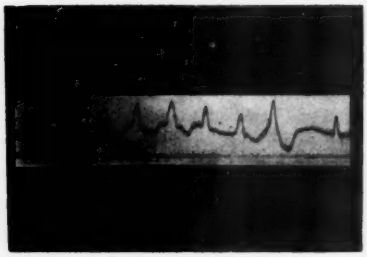

Fig. 2

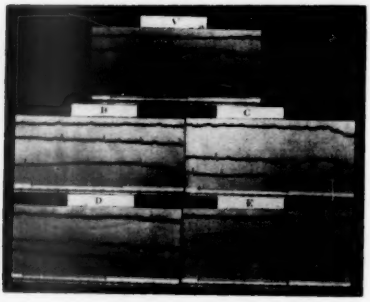

Fig. 1. See text

Fig. 2. ECG. of bilateral open chest under 4 cm. of water constant positive pressure. A: before op., B: 10 min., C: 30 min., D: 1 hour, E: 2 hours after bilateral open chest.

Fig. 3

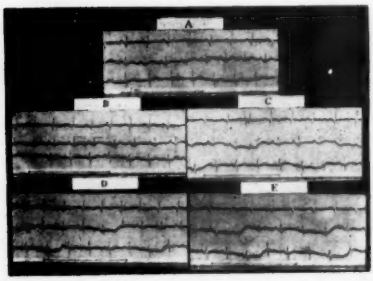

Fig. 4

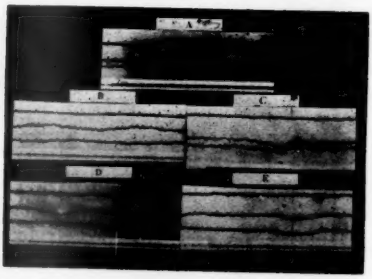

Fig. 3. ECG. of bilateral open chest under 8 cm. of water constant positive pressure. A: before op., B: 10 min., C: 30 min., D: 1 hour, E: 2 hours after bilateral open chest.

Fig. 4. ECG. of bilateral open chest under 16 cm. of water constant positive pressure. A: before op., B: 10 min., C: 30 min., D: 1 hour, E: 2 hours after bilateral open chest.

outside normal more than the standard error. *QT interval* became short in all cases except No. 7 after 30 minutes, 2 hours, respectively. None observed value lay outside the range of the standard error of the calculated mean. *Mean QRS electrical axis:* Without exception no change was noticed as in the cases of 4 cm of water positive pressure. *Pwave:* Pulmonary P was observed in all cases. *T wave:* Slightly flat in each lead.

(4) In case of 16 cm. of water constant positive pressure (see Fig. 4, Table III), *RR interval:* This was prolonged in all cases. Those which lay outside the normal limits were in No. 3 after 2 hours, in No. 14 after 2 hours and in No. 15. *PQ interval* prolonged in all cases except in No. 3 after 30 minutes and in No. 15 after 10 minutes. *QT interval:* All cases

TABLE II

ECG. of Bilateral Open Chest Under 8 cm. of Water Constant Positive Pressure

No. of case	Height of wave and time interval of waves / Course of time	P (m.m.) I	P (m.m.) II	P (m.m.) III	Q I	Q III	R I	R III	S I	S III	T I	T II	T III	● P (sec)	P—Q Observed value	P—Q Calculated value	Q—T Observed value	Q—T Calculated value	T—P	R—R	Mean QRS electric axis
No. 7	Before op.	1.8	1.0	0.8	4.1	0	6.5	7.8	0	6.5	0	1.0	1.8	0.029	0.073	0.068	0.137	0.140	0.041	0.249	+44°
	10 min. after open chest	1.2	2.0	1.8	3.5	0	7.3	6.4	0	5.9	0	1.3	1.6	0.029	0.068	0.067	0.133	0.139	0.050	0.244	+37
	30 min. " "	0.9	2.2	2.0	3.2	0	6.5	5.1	0	5.8	0.9	1.1	1.4	0.037	0.072	0.068	0.152	0.145	0.044	0.264	+41
	1 hour " "	0.9	2.3	1.9	3.6	0	7.4	6.0	0	5.0	0.6	1.2	1.6	0.030	0.072	0.067	0.131	0.139	0.044	0.242	+41
	2 hours " "	1.1	2.3	1.9	4.0	0	8.2	5.5	0	4.5	0	1.6	1.3	0.030	0.067	0.069	0.161	0.147	0.044	0.272	+41
No. 9	Before op.	1.5	2.0	2.0	0.5	0	2.1	5.1	1.9	7.4	2.1	4.5	4.0	0.031	0.077		0.151		0	0.225	−57°
	10 min. after open chest	0	2.4	2.5	0.6	0	1.8	2.9	0	5.5	1.0	2.3	2.5	0.031	0.089		0.131		0	0.223	−50
	30 min. " "	1.0	4.0	3.0	0	0	2.1	3.5	1.6	7.9	1.0	3.5	3.0	0.027	0.083		0.169		0	0.255	−61
	1 hour " "	0.5	2.0	2.7	2.2	0	3.0	3.8	0	5.4	2.0	2.5	0.6	0.030	0.073		0.153		0	0.226	−60
	2 hours " "	2.0	2.9	2.6	1.8	0	2.8	6.0	2.0	6.4	1.6	3.5	2.0	0.028	0.071		0.154		0	0.225	−60
No. 10	Before op.	0.5	2.5	1.7	0.5	0	1.9	5.4	0	3.3	1.0	3.1	2.7	0.031	0.053		0.183		0	0.234	+68°
	10 min. after open chest	0	2.0	1.9	1.0	0.2	2.1	3.1	0	0.7	0	2.7	2.6	0.027	0.046		0.152		0	0.199	+71
	30 min. " "	0	2.6	2.7	1·5	0.1	4.3	2.3	0	0.1	0	2.8	2.6	0.027	0.051		0.153		0	0.207	+58
	1 hour " "	0	2.5	1.0	2.3	0	4.2	4.8	0	2.2	0	3.0	2.4	0.029	0.059		0.186		0	0.248	+67
	2 hours " "	0	2.4	2.4	3.0	0.5	4.8	4.3	0	2.1	0	2.8	2.5	0.026	0.053		0.193		0	0.251	+65
No. 11	Before op.	1.2	3.1	2.7	3.4	0	3.6	8.0	0	8.1	0	4.4	4.2	0.032	0.061	0.068	0.158	0.143	0.038	0.254	−16°
	10 min. after open chest	1.4	4.2	3.5	2.2	0	3.5	8.0	0	9.0	0	4.2	3.9	0.028	0.053	0.065	0.138	0.136	0.040	0.231	−16
	30 min. " "	1.4	4.2	3.2	3.0	0	4.2	8.1	0	9.0	0	3.9	3.2	0.029	0.053	0.065	0.144	0.135	0.027	0.226	−13
	1 hour " "	1.5	3.5	3.1	2.1	0	4.7	7.9	0	8.1	0	3.6	3.4	0.028	0.055	0.065	0.141	0.135	0.030	0.227	−10
	2 hours " "	1.1	2.9	2.0	3.6	0	5.0	9.0	0	10.0	0	2.7	2.5	0.029	0.053	0.064	0.137	0.133	0.027	0.220	−16

Table III

ECG. of Bilateral Open Chest Under 8 cm. of Water Constant Positive Pressure

No. of case	Height of wave and time interval of waves / Course of time	P (m.m.) I	P (m.m.) II	P (m.m.) III	Q I	Q III	R I	R III	S I	S III	T I	T II	T III	P (sec)	P—Q Observed value	P—Q Calculated value	Q—T Observed value	Q—T Calculated value	T—P	R—R	Mean QRS electric axis
No. 3	Before op.	1.0	1.3	0	1.0	0	6.8	2.0	0	1.1	2.0	1.8	±	0.038	0.058	0.061	0.132	0.124	0.002	0.191	+38°
	10 min. after open chest	2.5	2.9	2.6	1.0	0	6.5	3.0	0	1.6	1.6	1.5	1.2	0.044	0.064	0.064	0.147	0.131	0.044	0.213	+42
	30 min. " "	2.0	2.5	2.0	0.6	0	8.0	6.0	0	2.3	0.5	±	±	0.025	0.045	0.067	0.170	0.141	0.025	0.246	+47
	1 hour " "	1.9	2.8	3.0	1.8	0	8.0	6.0	0	4.0	±	±	±	0.025	0.063	0.071	0.177	0.147	0.025	0.272	+44
	2 hours " "	2.3	1.8	2.0	1.0	0	8.9	7.0	0	2.0	0	0	0	0.031	0.096	0.079	0.225	0.178	0.077	0.395	+54
No. 13	Before op.	0.8	2.0	1.8	2.0	0	3.6	4.1	0	1.8	0	2.6	2.2	0.026	0.053	0.063	0.112	0.129	0.046	0.211	+65°
	10 min. after open chest	0	2.0	1.9	0.2	0	3.0	2.9	0	1.0	0	1.0	1.0	0.030	0.071	0.067	0.149	0.140	0.036	0.250	+70
	30 min. " "	0	2.5	1.4	1.8	0	3.1	3.6	0	2.1	0	1.8	1.2	0.027	0.063	0.069	0.164	0.146	0.039	0.267	+59
	1 hour " "	0	2.5	1.8	0.6	0	1.4	3.0	0	1.3	1.0	1.5	1.0	0.028	0.064	0.069	0.159	0.146	0.044	0.268	+70
	2 hours " "	0	1.7	1.2	0.8	0	2.2	2.7	0	0.9	0	1.5	1.0	0.025	0.071	0.071	0.152	0.152	0.062	0.289	+65
No. 14	Before op.	1.8	2.0	1.9	1.8	0	6.0	4.5	0	6.7	0	2.0	2.4	0.029	0.051	0.060	0.132	0.124	0.020	0.196	− 2°
	10 min. after open chest	1.0	1.8	1.0	1.0	0	6.0	2.0	0	5.5	0	0.5	0.5	0.034	0.069	0.064	0.102	0.129	0.038	0.210	− 2
	30 min. " "	1.2	1.6	1.8	1.1	0	5.8	2.0	0	5.0	0	0.5	0.5	0.025	0.063	0.065	0.130	0.135	0.036	0.231	− 7
	1 hour " "	0	1.2	1.0	1.5	0	5.6	2.0	0	5.5	0	0	0	0.021	0.071	0.067				0.250	− 6
	2 hours " "	1.2	1.4	0.9	0.5	0	4.4	0.5	0	2.7	0.6	0.5	0	0.031	0.082	0.072	1.172	0.160	0.068	0.321	− 4
No. 15	Before op.	0.8	1.8	1.5	0.5	0	3.2	3.8	0	3.0	2.3	3.0	3.0	0.020	0.074	0.065	0.135	0.136	0.022	0.233	+43°
	10 min. after open chest	1.0	1.2	0	0	0	2.4	5.0	0	3.6	1.7	2.5	2.0	0.012	0.071	0.092	0.272	0.222	0.272	0.614	+50
	30 min. " "	0	0.8	1.2	0	0	2.0	3.0	0	2.0	0	1.2	1.8	0.012	0.076	0.305	0.230	0.312	0.910	1.213	+49
	2 hours " "	1.0	0.8	0	1.0	0	2.0	4.0	0	3.0	1.8	0.8	2.0	0.019	0.096	0.306	0.493	0.313	0.636	1.221	+49

except No. 14 after 10 minutes showed prolongation. Those which were outside the normal limits were in No. 15 and in No. 3 after 2 hours. The measured values lay outside the range of the standard error of the calculated one was noticed in No. 3 after 30 minutes, 1 hour, 2 hours and in No. 15 after 10 minutes, 2 hours. *Mean QRS electrical axis:* The same results was obtained just as in the case of 4 cm of water positive pressure except in No. 3. *P wave:* Those which showed pulmonary P were No. 13 after 1 hour, 2 hour, and No. 3. *T wave* became flat in all leads without exception.

Discussion

It is probable that the electrical axis of heart will change by open chest. Judging from the experimental results described above, in many cases, the change of mean QRS electrical axis during open chest was within 10° and the type of electrical axis deviation did not change throughout. The chief changes noticed in E.C.G. were in the duration of RR-, QT interval and the heights of P and T waves. The most probable cause of the influences upon heart by open chest is hypoxia. Bibliographically, electrocardiographic findings concerning hyposxia are as follows: In cases of hypoxemia in dogs, according to W.C. Randall[5], a slight decrease in PR interval, QRS deflection and duration of electrical systole, respectively. C.T. Burnett[6] reports an early and prompt depression of T waves, especially of T_3. According to Greene and Gilbert,[7] in case of hypoxemia of slight degree, the duration of RR interval decreases and T wave becomes markedly flat. When hypoxemia becomes more pronounced, the heart beat weakened and eventually the dissociation occurs. Borgard[8] says that the first phase of tachycardia in hypoxemia is due to the decrease in vagal tone, and the second phase of bradycardia is due to the excitation of the vagal center. In the most severe hypoxemia, the heart itself suffered and exhibits disturbances in rhythm such as defect of conduction. According to experiments with the heart-lung preparation by M.B. Kountz and M. Hammonda,[9] general cardiac asphyxia by 50% oxygen makes the heart beat slower and T waves progressively flat in all leads. In lower percentage of oxygen, T wave becomes inverted; under 25% of oxygen, the RT interval changes and ST_3 elevates. Dietrich[10] says that if the amount of blood streem flowing coronary artery is decreased to one third of the normal, asphixic T, at least isoelectric T or inverted T appears.

W.B. Kountz[11] observed the relation of mean QRS electrical axis with T: T_1 and T_2 become low and T_3 high in left axis deviation, while T_1 and T_2 become high and T_3 low in right axis diviation.

Reviewing my experiments, the change of mean QRS electrical axis

by operative procedure is not so great as to cause change of the type of axis deviation and the hights of T wave decrease in all leads and are usually accompanied with prolongation of RR and QT interval. It may be permitted to say that the change of T is not due to the change of mean QRS electrical axis, but to a want of oxygen. In case of 8 cm of water constant positive pressure breathing, the flatness of T is very slight and the duration of RR-, PQ-QT interval decreases but still remains within the normal limits. This seems to be due to a slightness of hypoxia which doesn't cause coronary insufficiency. In cases of both 4 cm. and 16 cm. of water positive pressure, the flatness of T is so distinct as to become isoelectric T. Furthermore, in many cases, the duration of RR and QT interval lie over the normal limits and the measured value of QT interval is prolonged more than the standard error after open chest, while they lay within the normal limits before open chest. This fact shows that hypoxia in the former condition may have cause some coronary insufficiency in cardiac muscle. Pulmonary P appears in most cases during 8 cm and 16 cm of water constant positive pressure breathing. This suggests that the resistance in lung circulation has increased.

When constant positive pressure breathing is applied the carbon dioxid in blood accumulates. In my experiment, however, it was washed out by insufflating a good deal of oxygen for several minutes, by the positive pressure increased about 1 cm of water, each time before taking pictures of E.C.G. With such a procedure, the carbon dioxide content in blood returns to a level of almost normal as verified by the experiment of Horiuchi[22]. Therefore, the influence of carbon dioxide may be excluded.

Summary

The pattern of E.C.G. during bilateral open chest under various degree of positive pressure was studied in 12 rabbits.

1. *RR and QT interval:* In all cases, these values lay within the normal limits before operation prolonged by open chest under 4 cm. and 16 cm. of water constant positive pressure and in over half of all cases these values changed to outside the normal limits. Over half of the observed values of QT interval lay outside normal range of the standard error of the calculated mean. Most of them become short under 8 cm. of water constant positive pressure and lay within the normal limits.

2. *P wave:* Pulmonary P appeared under 8 cm. and 16 cm. of water constant positive pressure.

3. *T wave:* In each lead it became remarkably flat, especially T_2, under 4 cm. and 16 cm. of water constant positive pressure. It was slightly flat under 8 cm. of water positive pressure.

Conclusion

By the pattern of E.C.G., hypoxia appears in rabbits, during bilateral open chest under any degree of constant positive pressure. Hypoxia is very slight under 8 cm. of water constant positive pressure and the pattern of coronary insufficiency can't be observed, but it is obviously observed under both 4 cm. and 16 cm. of water constant positive pressure. In other words, if an adequate positive pressure relatively to the width of the wound of chest wall is applied a very slight degree of hypoxia appears which will not bring about coronary insufficiency. Coronary insufficiency will occur, by either higher or lower positive pressure than adequate one.

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An Experimental Research of Bilateral Open Chest

II. Oxygen Saturation of Blood during Bilateral Open Chest in Rabbits

By

Yoshinobu Ishikawa
(石川義信)

(From Prof. Katsura's Surgical Clinic, Faculty of Medicine, Tohoku University, Sendai)

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From the results of the former experiments as reported in the preceding part of this series of communication, it became known that during bilateral open chest under constant positive pressure, even in the best condition, the hypoxia in cardiac muscle appeared. The following experiments were carried out to find out the changes in blood oxygen saturation to elucidate the mechanism of the hypoxia during open chest.

Method

Animals, as well as the procedure of anesthesia and administration of positive pressure oxygen or air used for this experiment were the same as those used in the former experiment. Firstly, the chest was opened unilaterally by an incision made from posterior axillar line to sternal edge in the 6th intercostal space under atmospheric pressure. Secondly, both pleural cavities were opened by an incision made on the contralateral chest just in the same manner as above mentioned under 8 cm. of water positive pressure. Arterial blood was withdrawn from A. femoralis and venous blood from right ventricle, 0.5 c.c. at each time. They were collected before operation, 10 minutes after unilateral open chest, 10 minutes, 1 hour, and 2 hours after bilateral open chest, respectively. Oxygen saturation of the blood withdrawn from the vessels was determined by photocells.[1] The same kind of experiment was carried out also 4 cm. of water constant positive pressure of oxygen as well as under 8 cm. of water positive pressure of air.

Results

(1) Insufflation with oxygen under 8 cm of water constant positive pressure (see Table I, A): The oxygen saturation of arterial blood showed

the lowest value (average 83.8%) during unilateral open chest under atmospheric pressure except No. 4 and No. 5. But it returned closely to the normal value (average 93.5%) 10 minutes after bilateral open chest, attaining rather to a higher value than the normal (average 97.2%) after 1 hour to 2 hours. In mixed venous blood, the oxygen saturation was lowest (average 32.5%) 10 minutes after bilateral open chest and though it gradually rose, it remained lower than normal throughout. Consequently, the difference of oxygen saturation between arterial and venous blood was always higher than normal, being maximum (average 61.0%) 10 minutes after bilateral open chest. Therefore, the rate of

TABLE I

Oxygen Saturation of Blood during Open Chest

A: Insufflated with Oxygen under 8 cm. of Water Constant Positive Pressure

Figures experess percentages.

No. of case	Course of time / Blood	Before operation	10 min. after unilateral open chest	10 min. after bilateral open chest	1 hour after bilateral open chest	2 hours after bilateral open chest
No. 4	Arterial blood	96.5	87.6	69.3	95.1	98.5
	Venous blood		42.3	31.5	75.1	56.8
No. 5	Arterial blood	97.6	85.2	99.2	100.0	98.9
	Venous blood					
No. 6	Arterial blood	97.6	88.9	99.8	99.9	100.0
	Venous blood		33.3	20.2	23.9	23.8
No. 7	Arterial blood	95.8	86.7	98.6	100.0	100.0
	Venous blood		59.5	25.7	68.9	71.8
No. 9	Arterial blood	97.5	100.0	95.3	100.0	99.3
	Venous blood		51.9	38.0	79.6	60.8
No. 11	Arterial blood	97.6	86.5	91.3	97.2	99.8
	Venous blood		58.2	30.2	59.9	70.2
No. 13	Arterial blood	98.1	71.0	49.6	85.0	89.0
	Venous blood		35.0	49.6	52.0	62.2
No. 15	Arterial blood	94.7	64.4	100.0	100.0	98.9
	Venous blood		55.2		27.9	60.2
Average	Arterial blood	96.8	83.8	93.5	97.2	98.0
	Venous blood		46.4	32.5	52.4	57.0
The rate of oxygen usage			44.6	65.2	46.0	41.8

B: Insufflated with Oxygen under 4 cm. of Water Constant Positive Pressure

No. of case	Course of time / Blood	Before operation	10 min. after unilateral open chest	10 min. after bilateral open chest	Before death (time after bilateral open chest)
No. 10	Arterial blood	97.8	88.5		44.4 (8 min)
	Venous blood		69.8		42.4
No. 12	Arterial blood	97.2	80.1	72.5	49.9 (30 min)
	Venous blood		54.5	19.5	20.5
No. 14	Arterial blood	94.9	75.0		55.8 (9 min)
	Venous blood		29.8		30.9
No. 17	Arterial blood		87.2	94.6	43.6 (20 min)
	Venous blood		63.1	31.1	20.9

C: Insufflated with Air under 8 cm. of Water Constant Positive Pressure

No. of case	Course of time / Blood	Before operation	10 min. after unilateral open chest	10 min. after bilateral open chest	1 hour after bilateral open chest	2 hours after bilateral open chest
No. 2	Arterial blood	97.5	85.5	75.5	72.6	74.8
No. 3	Arterial blood	94.8	87.2	70.8	86.1	75.1
Average	Arterial blood	96.3	86.4	73.2	79.4	75.0

oxygen utilization was always greater than normal; its value decreased in the following order: 10 minutes and 1 hour after bilateral open chest, 10 minutes after unilateral open chest and 2 hours after bilateral open chest.

(2) Insufflation with oxygen under 4 cm. of water constant positive pressure (see Table I, B): Almost all animals died 10 minutes to 20 minutes after bilateral open chest. The oxygen saturation just before death decreased remarkably.

(3) Insufflation with air under 8 cm. of water constant positive pressure (see Table I, C): The oxygen saturation of arterial blood was greater in unilateral open chest than in bilateral open chest. Farthermore, it was always lower than normal during open chest.

Discussion

Yugaya[2] who determined arterial oxygen 2 hours after bilateral open chest under constant positive pressure by van Slyke's method reported that the oxygen saturation was decreased. He used air for in-

sufflation. C. Krafoord[3] reported that the oxygen saturation of arterial blood during bilateral open chest under constant positive pressure of oxygen remained normal. The results of my experiment were the same as the authors above. It may be enhanced oxygen partial pressure in the alveolus by insufflating with oxygen under positive pressure.

Anyway, the oxygen saturation of arterial blood is normal under 8 cm. of water constant positive pressure of insufflated oxygen when pleural cavities are opened, and anoxic hypoxia doesn't occur.

Under 8 cm. of water constant positive pressure erythrocyte count and hemoglobin content were also determined. The results were quite in accord with those by Izumiyama.[4] Hemoglobin showed no great change, while erythrocyte count decreased by about 9%. (see Table II). But this decrease might be partially due to withdrawal of blood samples. But it doesn't seem to play any important role in the production of hypoxia, i.e. hypoxia is not of anemic type. As shown in Table 5, the oxygen saturation of arterial blood is normal and the rate of utilization of oxygen is remarkably high. According to Moor,[5] furthermore, cardiac output decreases by bilateral open chest. Therefore, stagnant hypoxia plays the most important role.

In case of 4 cm. of water constant positive pressure, the remarkable

TABLE II

Erythrocyte Count and Hemoglobin Content during Bilateral Open Chest under 8 cm. of Water Constant Positive Pressure

No. of case	Course of time / Blood	Before operation	10 min. after bilateral open chest	1 hour after bilateral open chest	2 hours after bilateral open chest
No. 5	Erythrocytes	564	482	430	388
	Hemoglobin (%)	85	92	92	92
No. 6	Erythrocytes	469	402	484	444
	Hemoglobin (%)	92	90	97	92
No. 7	Erythrocytes	534	725	784	452
	Hemoglobin (%)				
No. 9	Erythrocytes	527	780	454	497
	Hemoglobin (%)	111	106	98	102
No. 11	Erythrocytes	647	639	425	666
	Hemoglobin (%)	108	104	86	95
Average	Erythrocytes	548 million	615 million	515 million	489 million
	Hemoglobin (%)	98	98	93	95

decrease of oxygen saturation of arterial blood through out the bilateral open chest indicates the presence of anoxic hypoxia. Under 4 cm. of water constant positive pressure, the animals didn't die as shown in experiments of the 1st report, but died in the experiments of 2nd report. This might be due to the withdrawal of blood.

Summary

In 14 rabbits the oxygen saturation of arterial as well as mixed venous bloods during bilateral open chest under both 4 cm. and 8 cm. of water positive pressure was determined.

(1) The oxygen saturation of arterial blood during bilateral open chest under 8 cm of water positive pressure was normal or above normal by insufflating with oxygen, while the utilization of oxygen by tissues increased.

(2) When air was insufflated, the oxygen saturation of arterial blood was lower in bilateral open chest than in unilateral open chest under atmospheric pressure.

(3) The oxygen saturation of arterial blood during bilateral open chest under 4 cm. of water positive pressure, distinctly decreased.

Conclusion

In case insufflated with oxygen under positive pressure adequate to the width of the wound of chest wall, not the anoxic anoxia but the stagnant anoxia seems to play the chief role in the production of oxygen deficiency in the cardiac muscle.

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An Experimental Research of Bilateral Open Chest

III. The Influence of the Degree of Constant Positive Pressure upon the Cardiac Output

By

Yoshinobu Ishikawa
(石川 義信)

(From Prof. Katsura's Surgical Clinic, Faculty of Medicine, Tohoku University, Sendai)

(Received for publication, December 14, 1951)

The preceding I. and II. reports showed the presence of the want of oxygen in cardiac muscles in cases of bilateral open chest under constant positive pressure breathing. In case of 8 cm. of water constant positive pressure, however, the E.C.G. revealed itself least abnormal. Probable the coronary insufficiency appears more easily under 4 cm. and 16 cm. of water constant positive pressure, respectively.

The following experiments were performed for the purpose of clarifying the cause of coronary insufficiency in case of 16 cm. of water constant positive pressure.

Method

Animals for experiment, the method to open chest, anesthesia and the way to apply positive pressure were almost the same as used in the experiments of the 1st report. The cardiac volumes in systole and diastole were recorded by the cardiometer of Henderson and Barringer.[1] At the same time, the degree of positive pressure was recorded. The systolic cardiac output was measured by the down strokes of the curve, its sum total in one minute showed the minute volume by both ventricles. These values were determind under the condition when the positive pressure from 4 cm. to 8 cm, 12 cm. and 16 cm. of water positive pressure, respectively. The value for 4 cm. of water constant positive pressure was made as the standard and the values under 8 cm, 12 cm. and 16 cm. of water positive pressure respectively, were compared with the standard.

Result

The minute volume under positive pressures, *measured* 15 *minutes*

after the chenge of pressure, increased in No. 16 and No. 12 under 8 cm. of water constant positive pressure, and remained almost the same in other cases; the average of the cardiac output under 4 cm, 8 cm, 12 cm. and 16 cm. of water constant positive pressure was 100%, 97.9%, 82.1% and 76.5%, respectively. The systolic output under 4 cm, 8 cm, 12 cm. and 16 cm. of water positive pressure was 100%, 93.6%, 71.2% and 60.5% respectively. The degree of decrease in systolic output was found higher than that in minute volume.

Minute volume, *just after the increase of pressure*, decreased in No. 2, No. 6 and No. 3 under 8 cm. of water positive pressure. Most of the other cases showed the same tendency. That is, the average of 5 cases 100%, 85.5%, 104% and 105.5% under 4 cm, 8 cm, 12 cm. and 16 cm. of water positive pressure. All increased except in 8 cm. of water constant positive pressure. The systolic output increased with the increase in positive pressure except in case of 8 cm. of water positive pressure, where the increase was not so great as in minute volume. The average was 100%, 89.1%, 96.0% and 101.0% under 4 cm, 8 cm, 12 cm. and 16 cm. of water positive pressure, respectively. (see Fig. I. Table I).

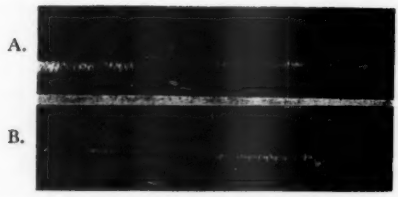

Fig. 1. Volume curves of two ventricles using cardiometer during bilateral oepn chest.
A: 15 min. after changes of pressure.
B: Immediately after changes of pressure.

Discussion

By cardiometer, combined stroke volume of two ventricles is measured. Reviewing the studies of Eyster,[2] Boyd[3] and Cahoon[4] concerning the effects upon the heart of the inflation of lungs during open chest under atmospheric pressure the combined stroke output decreased remarkably in inspiration, i.e. when lung was inflated. According to Boyd,[3] this decrease, in part or entirely, may be attributed to the decrease of stroke output of the left ventricle. G. H. Humphrey[5] determined cardiac output (in this case the cardiac output of the right ventricle) and concluded that it decreased compared with the increase of pressure. O. F. Opdye[6] reported that both the right and the left atrial pressures increased by pulmonary inflation. Duomarco J.[7] said that when the output of right

TABLE I

Cardiac Output During Bilateral Open Chest under various Degree of Positive Pressure

No. of case	Pressure (cm. of water)	Minute volume %	Stroke volume %
15min. after the changes of pressure			
N. 7	4	100.0	100.0
	8	76.4	63.1
	12	54.8	47.1
	16	40.7	37.1
N. 8	4	100.0	100.0
	8	96.4	96.0
	12	85.1	85.0
	16	99.9	97.0
N. 15	4	100.0	100.0
	8	90.8	90.0
	12	99.8	86.6
	16	88.7	67.7
N. 16	4	100.0	100.0
	8	100.3	100.4
	12	94.7	94.5
	16	83.4	85.8
N. 18	4	100.0	100.0
	8	85.5	80.6
	12	80.9	78.4
	16	78.4	77.4
N. 19	4	100.0	100.0
	8	96.0	96.1
	12	89.0	81.8
	16	79.8	79.2
N. 21	4	100.0	100.0
	8	137.8	128.9
	12	70.4	71.9
	16	68.2	70.0
Average	4	100.0	100.0
	8	97.9	93.6
	12	82.1	71.2
	16	76.5	60.5
Just after the changes of pressure			
N. 2	4	100.0	100.0
	8	83.6	67.2
	12	96.1	99.0
	16	102.1	105.0
N. 3	4	100.0	100.0
	8	96.5	94.3
	12	111.3	98.1
	16	111.3	94.3
N. 4	4	100.0	100.0
	8	100.8	90.9
	12	105.2	93.1
	16	102.4	103.6
N. 5	4	100.0	100.0
	8	117.2	101.0
	12	111.8	101.0
	16	109.0	99.0
N. 6	4	100.0	100.0
	8	94.0	92.1
	12	97.5	89.8
	16	102.3	102.3
Axerage	4	100.0	100.0
	8	98.5	89.1
	12	104.3	96.0
	16	105.5	101.0

ventricle was made artificially constant, circulatory resistance in lungs increased by pulmonary inflation and the pressure of A. pulmonalis became enhanced. Cloetta[8] said that when the lungs were inflated over a certain degree, the diameter of vessels became smaller, consequantly the resistance increased. According to my experiment, the cardiac output increased just after the pressure was increased, except in cases where the pressure was increased from 4 cm. to 8 cm. of water. It may be explained at least in parts in the following way. The lungs of rabbits under 4 cm. of water positive pressure were collapsed so much as the lower margins of lungs became hardly visible just below the wounds for open chest. In case of 8 cm. of water positive pressure, the lungs were inflated, and came to get contact with the chest wall; in case of 16 cm. of water positive pressure, the lungs got so inflated that they projected out the wounds of chest wall. As the entire chest wall was not removed in my experiment, when the lung was inflated excessively, the pressure inside the thorax seemed to increase. When the pressure was increased from 8 cm. to 12 cm, and 12 cm. to 16 cm, respectively, the lung was inflated excessively over the normal and the pulmonary vascular bed was exposed to pressure from outside with the result that the blood was pushed into the left side of heart. Consequently, the blood inflow into the left heart increased momentarily, and eventually cardiac output increased. As the deflated lungs got inflated to the normal, this phenomen did not occur, when the pressure increased from 4 cm. to 8 cm. of water. According to Nakamura,[9] the blood volume contained in the lungs liver and spleen increases respectively, when the positive pressure increased. Caar and Essex[10] reported that the reflux of blood into V. cava inf. was checked under positive pressure. Considering those reports, the decrease in cardiac output under 12 cm. and 16 cm. of water positive pressure, respectively, 15 minutes after the increase of pressure may due to the pressure upon both heart and great afferent vessels by pulmonary inflation and the increase of resistance in pulmonary vessels, both of which lead to hinder the blood flow returning to heart. The cardiac output decreases when the pressure increases from 4 cm. to 8 cm. of water positive pressure. According to Wiggers[11] and Strughold[12] cardiac output increases in hypoxemia. Feldman[13] said that cardiac output increases in hypoxemia at first and if hypoxemia continued decreases. According to my experiments which was described in the 2nd report, anoxic hypoxia exists under 4 cm. of water positive pressure. This can be interpreted as the momentary increase in cardiac output.

Summary

The cardiac output as well as minute volume of both ventricles during bilateral open chest under various positive pressures were determined by cardiometer in 12 rabbits. The output increased, in general, just after the change of pressure from 8 cm. to 12 cm and 16 cm. of water positive pressures, but decreased 15 minutes later. When positive pressure changed from 4 cm. to 8 cm. of water the output decreased immediately after the pressure was changed, and still more 15 minutes later.

Conclusion

In case of high positive pressure, the occurance of coronary insufficiency is due to broken balance of demand and supply of oxygen to the heart by the decrease of cardiac output which is, in turn, caused by both the increased intrathoracic pressure by pulmonary inflation upon atrio-caval system and the increased resistance in pulmonary vessels.

As a whole, hypoxia appears during bilateral open chest in rabbits under any kind of constant positive pressure. But the applied positive pressure is adequate to the size of the wound of open chest, the hypoxia is so slight that no coronary insufficiency is braught about. This kind of hypoxia is chiefly stagnant hypoxia. Anoxic hypoxia appears under lower positive pressure than the adequate one and stagnant hypoxia increases under higher positive pressure than the adequate one. Both of these circumstances induce coronary insufficiency.

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Experimental and Clinical Studies on Dyes for Excretory Function Test of Liver

By

Tatuo Nakazima
(中島 達雄)

(From the Medical Clinic of Prof. T. Kurokawa, Tohoku University, Sendai)

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The dye for the excretory function test of the liver has been investigated by many authors, and methylenblue (MB), indigocarmine (IC), congo red (CR), phenoltetrachlorphthalein (PTCP), azorubin S (AS), rose bengal (RS) and bromsulphalein (BS) had been used as the dye for the test. Moreover, we[1] added recently bromphenolblue (BPB) to these as a new dye.

In this paper, I examined experimentally and clinically from various points of view all dyes except PTCP which could not come to my hands.

Experimental

Preliminary experiment

Excretion of the dye into the bile and urine in dogs

Female dogs weighing from 10 to 15 kg. were employed in the experiment. The urine was taken with a catheter, and the method introduced by McMaster and Elman[2] was adopted for the collection of bile. The experiments began following the disappearance of jaundice 4 or 5 days after making the bile-fistula, and 3 cc. of 1% solution was applied as an injected dose in all dyes.

The results of experiment are shown in Table I.

With CR and RB, about 90% of injected amount was excreted in the bile, but without excretion in the urine.

With BS, the greater part of the dye (77.3%) was eliminated in the bile and a small proportion (2.6%) in the urine.

The excretion of AS and BPB in the urine was larger than that of BS, showing 12.4 and 6.2%, respectively, and the percentage readings in the bile were 72.3 and 76.5%, respectively, which were almost equal to that of BS.

Moreover, I found the excretion of 42.9 and 51.1% of injected IC

TABLE I

Excretion of Dye into Bile and Urine in Dogs

Dyes	Excretion in bile		Excretion in urine	
	Appearance time (minutes)	Output during 5 hours (%)	Appearance time (minutes)	Output during 5 hours (%)
MB	8.0	15.5	5—10	1.0
IC	3.0	42.9	2.5	51.1
AS	17.5	72.3	5.0	12.4
BPB	17.0	76.5	9.0	6.2
BS	6.0	77.3	8.0	2.6
CR	20.0	89.8		(−)
RB	7.0	91.9		(−)

AS: azorubin S, BPB: bromphenolblue, BS: bromsulphalein, IC: indigocarmine, CR: congo red, MB: methylenblue, RB: rose bengal.

in the bile and urine, respectively, showing lower percentage in the bile and higher in the urine as compared with five dyes mentioned above.

Total excretion of MB in both bile and urine was 16.6%, showing only 1.0% in the urine.

Toxicity of the dye

Frogs (Rana nigromaculata) weighing from 15 to 30 g. were employed in the experiments which were carried out in October and November.

As is shown in Table II, the minimal lethal dose for a frog per 1 g.

TABLE II

Toxicity and Diffusion Degree

Dyes	Lethal Dose (mg.)	Diffusion Degree (cm.)
IC	4.0	3.0
AS	2.5	2.8
CR	2.5	1.9
BPB	1.5	3.1
RB	1.5	2.9
BS	1.0	3.0
MB	1.0	2.2

of body weight was 1.0 mg. with BS and MB, 1.5 mg. with BPB and RB, 2.5 mg. with AS and CR, and IC was most non-toxic showing 4.0 mg.

Diffusion degree

20 cc. each of 0.5% agar was put in test tubes with the same diameters and 1.0 cc. of 1% aqueous solution of each dye was put upon them. The degree of diffusion into the agar was measured after placing it for 24 hours in the incubator kept at 38°C.

As is shown in Table II, the degree of diffusion was between 2.8 and 3.8 cm. in IC, AS, BPB, RB and BS, but it was as low as 1.9 cm. in CR and 2.2 cm. in MB.

Influence of pH variation on the tone of colour and colorimetric reading

Solutions containing 10 mg. of each dye in one thousand and four thousand cc. of water and urine with various extents of pH were prepared, and the change of tone of colour and variation of the reading of colorimeter were observed in each solution. Moreover, I examined colorimetrically the decolourization of each after leaving them for 5 and 24 hours.

AS: Water solutions below pH 5.0 presented brilliant red colour and the constant reading in colorimeter, but, above pH 5.5 they showed orange colour and a reduction in the reading by half. The change of colour in urine was identical with that in water, though a slight decrease of the reading in colorimeter was found in solutions above pH 6.5.

BPB: Water solutions above pH 6.0 showed violet colour and the constant reading of colorimeter, while, it was difficult to determine it colorimetrically because of the appearance of reddish violet below pH 5.5. On the other hand, the tone of colour was constant in urine showing violet above pH 5.0, and the reading dropped colorimetrically below pH 5.0.

BS: Both water and urine solutions below pH 6.5 were colourless, and water above pH 7.5 and urine above pH 9.0 showed violet and a definite reading in colorimeter.

CR: Both water and urine solutions above pH 6.0 presented reddish orange colour and a definite reading colorimetrically, but it was difficult to determine them colorimetrically because of violet and dark brown colour, especially in urine below pH 5.5.

RB: Both water and urine solutions below pH 5.0 and 4.0, respectively, presented rosy red colour and a definite reading in colorimeter, though the tone of colour and the reading decreased remarkably below these pH values.

Furthermore, it was impossible to find decolourization after 5 and 24 hours in both water and urine solutions of each pH.

Excretion of the dye following disturbance of parenchymal and kupffer cells

Method

Female dogs were employed in the experiment as before.

Subcutaneous injection of 1.0 cc. of chloroform mixed with the same amount of olive-oil or of 0.6 cc. of 0.5% phosphorated oil per kg. of body weight was adopted in order to disturb parenchymal cells.

For the purpose of impairing Kupffer cells, 90–100 cc. of 1% saline solution of colloidal silver was injected only once or 40 cc. of 3% saline solution of India ink was injected intravenously once a day for over ten days.

The retention of the dye in the blood and elimination in the urine were estimated after the injection of 5 mg. of each dye per kg. of body weight to the large saphenous vein of a dog. The blood was taken out in 15 and 30 minutes, then every 30 minutes till 2 hours after the injection. Serum was obtained by centrifugation of the blood by adding 3.8% aqueous sodium citrate solution at the rate of 10% to the sample. Solution containing 10 mg. of the dye in 100 cc. of water was regarded as the concentration of 10%. The urine was taken by a catheter every 30 minutes after injection till the disappearance of the dye and the percentage reading of excretion for injected amount was measured.

Results

4–6 dogs were employed for the experiment with each dye and the estimation was done several times from the occurrence of disturbance till the recovery to the normal state.

A typical case in the stage of a remarkable disturbance is shown here.

AS: In 10 healthy cases, the retention in the blood was 6–12%, 2–6% and 0–2% at the end of 15, 30 and 60 minutes, respectively. The excretion in the urine ranged from 8.0 to 16.4 %,except in 2 cases in which it was 3.0 and 4.6%.

In the disturbance by chloroform, I found the retention of 20 and 10% at the end of one and two hours, respectively, while the excretion increased to 68.0%.

In the disturbance by phosphorus, the retention was 7 and 1% at the end of one and two hours, respectively, and the excretion was 2.8% showing a low reading which was due to the kidney disturbance resulting from the phenolsulphonphthalein test.

In the impairment by colloidal silver, the retention was 8 and 5% at the end of one and two hours, respectively, and the excretion in the urine was 41.9%.

BPB: In 10 healthy cases, the retention in the blood was 7–13%, 3–7% and 0–2% at the end of 15, 30 and 60 minutes, respectively. The total excretion in the urine ranged from 1.9 to 6.0%.

In the disturbance by chloroform, the retention of 40 and 10% were found at the end of one and two hours, respectively, and the excretion increased to 48.3%.

In the disturbance by phosphorus, the retention showed a slight increase, namely, 8 and 2% at the end of one and two hours, respectively, and no difference of the excretion in the urine was found in comparison with that before the disturbance.

In the impairment by colloidal silver, the retention and excretion showed a slight increase.

BS: In 8 healthy cases, the retention in the blood was 2–8%, 1–5% and 0–2% at the end of 15, 30 and 60 minutes, respectively, and the total excretion in the urine was below 0.5%.

In the disturbance by chloroform, the retention was higher than those with the first 2 dyes, showing 32 and 20% at the end of one and two hours, respectively. On the contrary, the excretion in the urine showed lower percentage than that with AS, namely, 18.0%.

In the impairment by colloidal silver, the retention was 10 and 5% at the end of one and two hours, respectively, and the excretion showed only a slight increase as compared with that before the disturbance.

CR: In 8 healthy cases, I found the retention of 40–46%, 20–30%, 8–10%, 3–13% and 0–5% at the end of each time given before. The reading was higher and longer time was required for the removal of the dye from the blood as compared with other dyes. No excretion in the urine was found.

In the disturbance by chloroform, the retention increased remarkably to 60 and 50% at the end of one and two hours, respectively, but only a trace of the colour was found in the urine.

In the impariment by colloidal silver, the same results as with chloroform were obtained, though the reading of retention was slightly low.

RB: In 9 healthy cases, the retention in the blood was 8–20%, 3–8%, 2–4% and 0–3% at the end of each time mentioned above. Little or no trace of the colour was found in the urine.

In the disturbance by chloroform, the retention as high as 35 and 30% were found at the end of one and two hours, respectively, while only a trace of the dye was found in the urine.

In the disturbance by phosphorus, I found the retention of 11 and 3% at the end of one and two hours, respectively. The excretion in the urine was the same as with chloroform.

In the impairment by colloidal silver, the similar retention was found

as with phosphorus and no excretion in the urine.

In the impairment by India ink, no remarkable difference was found between the results of experiments before and after the disturbance with all dyes, though the excretion in the urine slightly decreased as compared with those with AS and BPB.

Clinical experiment

Cases brought to my attention were 5 healthy persons and 22 cases of liver, gall-bladder and duct diseases, and AS, BPB, BS, CR and RB tests were performed with these cases.

Method

The following methods for determination were applied to each test.

In AS test Kanai's[8] method was adopted and the reading over 15% was accepted as pathological.

In BPB test 4.0 cc. of 0.5% solution was injected intravenously and the excretion over 11.0% in the urine was considered to be pahtological after Numasawa.[4]

In BS test 5 mg. per kg. of body weight was given intravenously using 5% solution of the dye. The standard of disturbance was reported by many researchers,[5][6][7] but I called the retention over 6 %in the blood stream at the end of thirty minutes abnormal after Israel and Reinhold.[5]

CR test was done after Unoura[8] who called the reading over 72.0 of the index abnormal.

In RB test Sawada's[9] method was adopted in which the retention of more than 0.3 mg. % at the end of 45 minutes after the injection of 1.5 mg. of the dye per kg. of body weight was said to be indicative of the liver disturbance.

Results

All tests showed the normal value in 5 healthy persons, among whom only one case showed a slight disturbance in CR test.

The disturbance rate of each test in patients was as follows:

Of 22 cases, 73 and 82% in AS and BS tests, respectively, of 21 cases, 81% in BPB, of 20 cases, 65% in CR and of 12 cases, 58% in RB test.

From these results, it is seen that BS and BPB tests showed the highest percentage of disturbance, and that AS and RB tests showed the lowest. No significant difference was observed between the disturbance rate of each dye from the statistical study.

Moreover, I found the interrelation between the disturbance in AS and BPB tests, AS and BS tests, AS and RB tests, BS and RB tests with

TABLE III

Clinical Application

No. of Case	Sex	Age	Diagnosis	AS		BPB		BS		CR	RB
				Output (%)	Disappearance time (hours)	Output (%)	Disappearance time (hours)	Retention in blood 30 Min.	Retention in blood 45 Min.	Index	Retention in blood (mg. %)
1	F	25	Healthy	13.5	5	8.5	5	3.9	2.5	69.7	
2	F	26	"	11.8	5	7.4	5	6.0	2.0	71.0	0.19
3	M	56	"	9.1	3	9.2	5	3.0	0	74.5	0.25
4	F	53	"	13.9	3	7.2	5	4.5	0	68.8	0.14
5	F	29	"	11.2	5	8.9	5	2.5	0	65.0	0.18
1	F	53	Cancer of liver	18.2	5	11.9	5	6.7	4.1	77.2	
2	M	57	"	42.4	23	44.2	23	45.0	40.0		0.66
3	M	59	"	46.1	11			25.0	22.5	79.4	0.51
4	M	41	Hepatitis	51.1	23	70.9	24	73.0	52.6	96.0	
5	F	24	"	53.4	24	65.6	24	80.0	73.6	73.2	
6	F	19	"	25.5	5	12.7	5	46.5	22.0	75.0	
7	M	48	"	20.8	5	12.2	5	8.0	6.0	68.4	0.38
8	M	26	Cirrhosis of liver	31.1	9	25.5	13	20.6	10.9	69.8	
9	M	63	"	36.4	11	23.8	11	45.0	35.0	85.8	0.60
10	M	20	Cholecystopathy	24.3	5	19.8	5	13.8	7.5	68.7	
11	F	51	"	13.0	4	9.4	5	4.5	0	68.2	0.30
12	M	58	"	13.8	4	14.5	5	4.0	2.0	90.0	0.18
13	M	21	"	11.2	5	11.9	5	2.0	0	69.3	0.13
14	F	33	"	17.1	5	14.7	5	22.5	20.0	87.0	0.40
15	M	40	"	9.9	3	8.6	4	2.5	0	69.2	0.16
16	M	34	"	15.5	5	11.2	5	6.5	2.5	71.4	0.33
17	M	47	Cancer of bile duct	52.9	24	48.4	24	52.2	29.6	85.7	
18	M	53	Cancer of pancreas	61.3	24	63.8	24	27.5	23.0	22.4	0.48
19	F	60	Cholelithiasis	58.6	24	71.9	24	46.4	31.2	86.2	
20	M	50	Weil's disease	5.4	4	4.3	5	8.0	4.7		0.27
21	M	43	Hepatic congestion	8.8	4	6.7	5	22.5	7.9	76.2	
22	M	26	Tumor of liver	16.4	5	13.6	5	6.6	3.7	74.4	

the statistical significance showing the risk-rate under 1.2%. There is, however, no relationship between the disturbance in CR test and all other tests.

No side action of importance was found in each dye, though only in one case a slight thrombophlebitis was presumed to have been caused after the injection of BS.

Summary

The excretory function of the liver has been tested clinically by the excretion of the dye in the urine or by the retention in the blood stream.

The following dye is suitable for these purposes as Nakagawa[10] suggested. The dye which is eliminated in the bile and not in the urine in most useful in the test by the retention in the blood stream, while, for testing by the urine, it is preferable that the greater part of the dye is excreted in the bile and only a small proportion in the urine in normal condition, but that an increased output and a prolonged duration in the urine are found when the liver is disturbed.

Moreover, it is most advisable for the test that the dye is stable and non-toxic for human body and presents a distinct tone of colour favourable in determining colorimetrically.

It is considered from the elimination in the bile and urine in dogs that MB and IC are unsuitable as the dye.

AS is stable and non-toxic and presents a brilliant red available for determination. In normal dogs, over 72% of the dye is excreted in the bile and about 10% in the urine, while the output in the urine increases remarkably with a moderate retention in the blood in the liver disturbance, especially by chloroform. I found the same results clinically as in the animal experiments, showing an ideal dye for testing by the urine.

As Numasawa[4] already stated, BPB is also applied for the same object as AS. Moreover, we[1] can distinguish by photoelectric colorimeter more exactly the difference of concentration in BPB as compared with AS, though it has double tone of colour which gives no great difficulty in determination.

BS has also the same tendency of excretion as AS in the normal condition of bile-fistula dogs. However, in the liver disturbance, especially by chloroform, the retention in the blood is more remarkable than the increase of excretion in the urine as compared with AS. Moreover, the dye presents a suitable tone of colour for determination, and I found high percentage of disturbance clinically. From these facts, BS is one of the most available in the test by the retention in the blood.

CR is useful in testing by means of blood, for the greater part of the dye is eliminated in the bile and there is no and a little trace of the colour in the urine in normal condition and the liver disturbance, respectively. However, not only high retention but also prolonged removal from the blood are observed in normal condition as compared with AS, BPB, BS and RB. Moreover, it is peculiar that a positive result is obtained in a healthy person and that no interrelation is indicated between the disturbances with CR and with other dyes in patients.

Considering from the toxicity and preliminary experiment in dogs, RB is preferable as the dye for the test by the blood as well as BS. However, the previous method is not so sufficient clinically, and it may be expected that RB will be utilized with the improvement of the method.

PTCP could not come to my possession, but Nakashima[11] reported this is unsuitable for the test from the fact that only 12% of injected amount is eliminated in the bile in a normal dog and it has side actions.

As above mentioned, the tone of colour and the reading of colorimeter are changeable according to pH variation in all dyes, and it is necessary to amend pH of the urine in AS test and serum in BS test to determine with constant colouring.

Conclusions

1. Methylenblue and indigocarmine is unsuitable as the dye for the excretory liver function test.
2. Azorubin S and bromphenolblue are most ideal for the test by the urine.
3. Bromsulphalein and congo red, especially the former, are suitable as the dye for the test by the retention in the blood stream, showing the highest percentage of disturbance clinically in bromsulphalein as compared with other dyes.
4. Rose bengal is also preferable, provided the improvement in the testing method is expected hereafter.

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Studies on Liver Function in Alloxan Diabetes

By

Sigemiti Tuda

(津田 重通)

(From the Mdical Clinic of Prof. T. Kurokawa,
Tohoku University, Sendai)

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It is an old recognized fact that diabetes mellitus has a close bearing on the metabolic function of the liver, often causing its disturbance. Recently minute investigations have been done on latent disturbance by various liver function tests, but the results arrived at by the researchers are not always in agreement. Reports in which influences of dietary treatment on liver function are pursued are very few.

On the other hand, many researchers acknowledged the pathohistological changes of liver in case of alloxan diabetes, but as shall be stated later, we have had no report giving satisfactory observations on liver function.

I made alloxan diabetes induced in rabbits and tried to inquire into the functioning of liver in its natural process and at the same time the effect upon the liver function of the standard diet, which had been given to some of the stricken rabbits.

Experimental

Method

Animals employed were adult male rabbits weighing 1.8–2.7 kg. when the experiment was begun. These animals were fed as much as they desired all through the period of experiment except for a short time of "standard diet." In good health they ate 130–150 g. of *tofukara* (bean-curd husk) and 80–100 g. of vegetables or greens per kg. of body weight per day, but 1.5–2.5 times as much when they suffered from diabetes. By the way the "standard diet" consists of 120 g. of *tofukara* and 60 g. of vegetables or greens per kg. per day.

Tests applied for liver function were sodium santonate test (S test) and azorubin S test (A-S test), the former being used as detoxicating test and the latter as excretory function test.

S test was applied after Takasugi-Miyamoto's method[1]; only to one same animal the same dosage of santonate as that administered in

the first test was always given regardless of the loss of its weight.

In A-S test always 2 cc. of 1% A-S solution was intravenously injected. The amount of this dye excreted in the urine after an hour was determined by using Dubosq's colorimeter and the percentage of the amount excreted to that injected was obtained.

After more than a week's acclimatization liver function tests were put to each animal to know its normal value. I obtained the average value of more than three successive tests where almost equal readings were got; then alloxan was administered.

To most of the animals a large dosage (190–200 mg. per kg. of body weight) and to a part a small dosage (100 mg. per kg.) of alloxan were intravenously injected each at a single time.

Blood sugar was determined through Fujita-Iwatake's method[2] in the morning fasting time of the subject.

For renal injury after injection examination was made by albuminuria and phenolsulfonphthalein test (PSP test) and when there was found any injury liver function tests were not carried out.

Results

1) Appearance of alloxan diabetes and their classification

Among the 32 rabbits to which alloxan had been administered, diabetes was observed to have appeared in 26 cases out of 28 with a large dose injected and 2 cases out of 4 given a small amount, the 4 others not responding.

It was 21 among these 32 that were with satisfaction employed for my observation of liver function. They are classified under the following 4 types for the convenience of consideration.

Type I (Cases 1–4): To this type belong those animals that suffered from serious diabetes, rapidly grew weaken, and died in the early stage of the disease. In all the cases alloxan was administered in large amount.

Type II (Cases 5–12): In these cases permanent diabetes was induced. All the rabbits of this type were given a large dose except Case 11 into which a small dose was injected.

Type III (Cases 13–17): Under this type those animals fall which presented transitory diabetes. Glycosuria, which had been found in all the cases of this type, disappeared within 3 weeks. In 4 cases a large, and in 1 case a small quantity of alloxan had been administered.

Type IV (Cases 18–21): This type consists of 4 cases in which alloxan found no response. Two were given a large amount and the other two a small amount.

2) Duration of renal injury after alloxan administration

In the group injected with small amount renal injury was detected in netither of the 4 cases including Case 11 of Type II, but in the other with large amount 13 cases out of 17 (76.5%), the 2 cases of Type IV included, gave albuminuria. Albuminuria was most remarkably found about 24 hours after alloxan administration, occasionally accompanied by macroscopic hematuria; then speedily better on towards recovery, and disappeared in 2–10 days (in most cases 5 days).

According to PSP test the duration of excretory dysfunction of the kidney was in all cases corresponding to that of presence of albuminuria; only three cases required 1–3 days more to be recovered after the disappearance of albuminuria.

To say in addition, incidental injury in the kidney seemed to have hardly anything to do with the diabetes or its severity.

3) Liver function

a) S test

Type I. In all cases the output of the dye (santogenin) decreased remarkably (i.e. lower than 16% of normal value) on the occurrence of diabetes, decreasing more and more towards 0 as time went and in the 3rd or the 4th week after injection the diabetic animals died.

Type II. As shown in Table I and Fig. 1, the curves of output fell sharply simultaneously with the appearance of diabetes in all cases except Case 12 which escaped satisfactory observation in its early stage, arriving at their lowest values (8.3–31.1% of normal value) in the 1st or 2nd week. The curves thereafter rose again and culminated about 4 weeks after the administration. The readings at their uttermost points, however, are considerably lower than normal values except in Case 6.

In chronic diabetes extending beyond the 5th week, the curves, in so far as some special controling treatment was not given, ran, generally in the sphere lower than normal level, almost in response to the severity of diabetes. In other words, the output increased towards normal level as diabetes improved (Cases 7, 8, 10 and 12), decreased off the level as it deteriorated (Cases 5, 6, 9 and 10), and further decreased towards 0 when the disease was critically serious (Cases 5, 6 and 9).

Type III. In all cases the output decreases and increases in a curve similar to that described by Type II. But in this type the lowest value is generally higher (18.3–61.1% of normal value) than in Type II, the curve not stopping rising about the 4th week till it reaches the normal level before the diabetes has been recovered.

Type IV. The curve of output of this type resembles that of Type III, but the lowest value reached in the 1st or 2nd week was generally far higher (32.9–77.1% of normal value) than that in Type III and re-

covery since then was expedite. Normal value was reached in all cases in the 2nd to 4th week.

TABLE I

Liver Function in Alloxan Diabetes

No. of cases	Type	Test*	Before alloxan (normal value)	After alloxan (weeks)								
				1	2	3	4	5	7	10	15	20
5	II	B S	103	460↓	352	292	295↓	270↓	320	418	425	186†
		A-S	26.1		28.3	28.2	26.5	26.0	25.9	25.6	29.1	19.0
		S	24	×	× 4	4	16	20	11	15	11	1
6	II	B S	100	386↓	342	292	352	352	331	205	†	
		A-S	24.2	23.0	25.5	25.6	24.6	24.9	23.8			
		S	81	×65	21	51	88	64	19	3		
7	II	B S	106	324↓	292	338	312	285	266	205	174	164
		A-S	28.5		24.5	30.4	31.3		30.2	29.1	29.4	30.2
		S	96	×32	25	41	15		30	32	40	64
8	II	B S	102	330↓	344	334	244	202	216	184	178	150
		A-S	27.6	29.3	32.5	30.3	25.4	26.7	30.2	30.6	32.5	32.2
		S	46	×10	29	20	22	28	36	53	29	29
9	II	B S	112	414	386	291	284		265†			
		A-S	28.7		29.7	27.1	23.8	23.7	26.7			
		S	253	×65	21	28	25	2	0			
10	II	B S	107	344	292	263	249	205	166	141	277‡	
		A-S	27.8			31.9	25.8	25.5	26.5	28.2	35.2	
		S	252	×49	×26	68	124	158	167	217	99	
11	II	B S	110	341	282	237	277	246	278	280	284	
		A-S	32.1	40.6	36.0	26.8	25.2	25.8	32.5		32.6	
		S	45	16	30	35	21	34	38	41	40	
12	II	B S	104	365	372	284	153			191	261	219
		A-S	27.7	31.5	34.7	29.8	27.5			27.2	27.8	
		S		×	5		36			37	32	62
13	III	B S	107	310	230	216	146	144	131			
		A-S	30.2	31.7	31.6	30.2	30.8	30.5	30.4			
		S	82	×15	22	31	43	57	81			
19	IV	B S	109	146	108	112	103					
		A-S	27.5	27.8	27.2	27.6	27.6					
		S	70		23	54	72					

* BS blood sugar (mg.%), A-S azorubin-S test (%), S sodium santonate test.
↓ insulin administration. × renal injury. † died of serious disease. ‡ died by accident.

b) A-S test

Type I. The percentage of output in the 1st or 2nd week increased

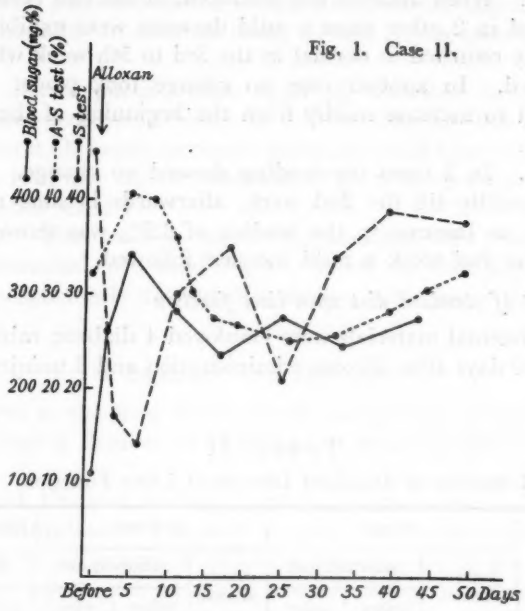

Fig. 1. Case 11.

in all cases over the normal value by 2–16%.

Type II. On the occurrence of diabetes reading of the percentage increased, as is shown in Table I and Fig. 1, except in Case 7, reaching the highest value (1–8.5% higher than normal) in the 1st or 2nd week, then decreased till it came to the lowest value almost in the 4th week. Here, the readings of Cases 5, 6 and 12 were directly restored to normal, while those of Cases 8, 9, 10 and 11 decreased to lower than the normal value, then gradually increasing again to normal by the 7th week. In other words, the lowest values of the former three were their respective normal values, while those the latter four arrived at were lower than normal. Case 7 alone, taking its own course, culminated in the 4th week and then decreased, still showing higher values than normal.

In the chronic diabetic stage Cases 5 and 10 showed a significant increase of reading when the diabetes was aggravated, but generally speaking, other cases, in spite of rather manifest diabetes persisting, presented most often normal values except for Cases 7 and 8 which still had readings higher than normal in their improving stage when glycosuria had disappeared. Further to say, Case 5 showed remarkably low reading, by far lower than normal, when the disease was serious.

Type III. When diabetes was produced, in one case (Case 13, Table I) a slight and in 2 other cases a mild decrease were exhibited of their readings; they returned to normal in the 3rd to 5th week when the diabetes improved. In another case no change took place. The other one continued to increase mildly from the beginning of the disease till after recovery.

Type IV. In 2 cases the reading showed no change. In another it decreased mildly till the 2nd week, afterwards became normal. In the other one an increase in the reading of 8.5% was shown in the 1st week, after the 2nd week a mild increase followed.

c) Effect of standard diet upon liver function

As experimental materials were employed 4 diabetic rabbits of Type II of 50 to 150 days after alloxan administration and 3 uninjected rabbits for control.

TABLE II

Influence of Standard Diet upon Liver Function

No. of cases	Duration of standard diet	S test			A–S test			Blood sugar mg.%	
		before alloxan	Standard diet		before alloxan	Standard diet		Standard diet	
			before	after		before	after	before	after
5	4	24	11	43	26.1	29.1	26.9	422	428
	9			71			21.2		329
6	4	81	8	31	24.2	23.8	18.0	302	268
	9			94					268
7a*	5	96	22	56	28.5	30.0	26.5	268	205
	15			83			26.5		205
7b*	3	96	64	64	28.5	28.4	28.0	174	93
	10			94			24.1		93
11a*	8	45	38	107	32.1	32.5	22.1	278	188
11b*	4	45	41	56	32.1	32.5	24.6	280	181
	11			108			23.4		181
22 †	10		70	76		23.5	23.7		
23 †	10		44	39		26.5	26.8		

* a and b signify the 1st and 2nd experiments respectively of the same animal.
† control case.

Effect of the standard diet on liver function was without any exceptions observed remarkably in diabetic cases, though not in control cases at all (Table II).

S test. Before putting them upon the standard diet their output had in all cases decreased, though in different degrees, but about the 4th day of the diet it obviously increased except the mild Case 7b, and between the 8th and 15th day the increase was so great as to exceed the normal value except for Case 7.

A-S test. The reading before the standard diet was begun was normal or increased to a mild degree. After the diet was given for 8-15 days it conspicuously decreased to show a value lower than normal in all cases.

Summary and Discussion

In respect to the liver lesions in the early stage of alloxan diabetes there have been a number of experimental results published by various researchers.

In several kinds of animals hepatic injury is often observed after alloxan administration. A report[3] is made that in this case the severity is very different according to different species. As to the relation between hepatic injury and dosage of alloxan Bailey[4] emphasizes that the larger the dosage that has been administered, the greater the toxic effect in all species. On the other hand, Lukens[5] reports " the incidence and severity of hepatic involvement could not be related to the dosage of alloxan."

To turn our eyes on the reports on hepatic injury accompanying the diabetes in rabbits, Hard et al.[6] observed no histological change after administering 50–100 mg. of alloxan per kg. of body weight. Bailey et al.[3] and Okamoto[7] observed slight degeneration with 200 mg. injected; Kendell et al.[8] reported fairly serious lesion caused by 125 mg. of alloxan per kg. In my observation of alloxan diabetes dysfunction of liver was noticed in all cases regardless of the dosage of alloxan. It was characteristic especially in Type II. The disturbance of liver in Type II was detected by both S test and A-S test, though slightly by the latter, simultaneously with the appearance of diabetes. This injury reached its maximum in the 1st or 2nd week, then gradually grew improved and about in the 4th week was at its minimum. In this stage A-S test proved the recovery of the excretory function of the liver to normal or inferred its hyperfunction, but S test showed the disturbance of detoxicating function was still somewhat serious.

On the other hand, in Type IV disturbance was discovered in all cases by S test and in half of the cases by A-S test. It generally dis-

appeared by the 4th week. This disturbance in Type IV was caused by alloxan alone, as there was no diabetes induced.

In respect to liver disturbance Type II and Type IV are found to hold parallel course in decreasing or increasing, especially through S test. But the disturbance in the former type is much severer and in the 4th week is cleanly told by S test to still persist, though by this time that in the latter has completely disappeared, and afterwards shows no great change. This comparison leads to conclude that the remarkable disturbance observed in Type II in the 1st or 2nd week had not been produced by alloxan alone, but with collaboration from diabetes and that the disturbance in the same type noticed after the 4th week was that caused by diabetes which was made exposed in consequence of disappearance of alloxan influences.

As for other types, Type I is the extreme case of Type II, where the serious diabetes made the liver dysfunction severe enough to cause the death of the stricken animals. Type III is, as it was, the abortive Type II. In this type disturbance shown by S test ran an intermediate course of Type II and Type IV to recovery, not stopping in the 4th week as in Type II, because there was improvement in diabetes in the preceding 2nd or 3rd week, and spending more time in returning to normal than in Type IV.

As for the liver function in chronic diabetes, influences of alloxan were, as stated before, gone out by about the 4th week. Therefore, the dysfunction of liver observed after the 5th week, was, unless complicated, caused by diabetes.

On the histological examination of rabbits Bailey et al.,[8] Duff et al.[9] and Okamoto[7] recognized no abnormality of liver, and Lewis et al.[10] told of the same plasma protein changes (i.e. a marked increase in the ß-globulin and decrease in albumin levels) in rabbits as in human diabetes.

According to the results of my observation on uncontroled alloxan diabetes, S test proved there almost always to be disturbance in all cases and there to be a certain relation between the severity of disturbance and that of diabetes. But in A-S test distrubance was detected only in half of the cases, the disturbance not always being related to the severity of diabetes.

As regards liver dysfunction of human diabetes Kaito[11] found positive result in 70% of 45 cases (54% of mild and 89% of serious cases) by S test and in 65% of 43 cases (there is no statistically significant difference between the mild and serious cases) by A-S test. Koga[12] reports positive S test in 45.5% of 22 cases, Nakagawa et al.[13] positive A-S test in 2 cases out of 4.

It gives us a deep interest to know that my experiment of alloxan diabetes has reached substantially the same result as of human diabetes.

Now for the effect of "standard diet" Kaito[11] examined by S test the influences of dietary treatment on liver function of human diabetics and observed improvement in 6 out of 13 mild and 6 out of 10 serious cases. I made researches as to the effect of standard diet upon liver function in alloxan diabetes by both S test and A-S test and not only found improvement in all cases, but obtained such results as to suggest hyperfunction of liver in most cases. This effect gradually strengthened till about 8 days from the beginning of the treatment, but thereafter seemed not to make any remarkable changes.

When the standard diet was followed by free diet, the effect was gone off almost in a week and the liver function resumed the level before the standard diet.

Conclusions

1. In the early stage of alloxan diabetes there was remarkable dysfunction of liver observed which was caused by diabetes and, further, strongly influenced by alloxan, influence of the latter acutely passing away.
2. Dysfunction of the liver in chronic alloxan diabetes was detected in all cases by santonin test and in half of the cases by azorubin S test.
3. Liver function in alloxan diabetes was remarkably improved by the treatment of standard diet.

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Studies on the Cardiac Rhythm of the Dog during Bilateral Thoracotomy and after Vagotomy and Esophagectomy

I. Studies on the Cardiac Rhythm of the Dog during the Bilateral Thoracotomy

By

Wataru Kojima
(兒島 渡)

(From the Surgical Clinic of Prof. S.-T. Katsura Faculty of Medicine, Tohoku University, Sendai)

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Introduction

It is well known by the clinical studies of Torigata and Osawa[1] and others that operations in chest opened unilaterally can be safely performed under the ordinary atmospheric pressure. Thoracic operations, however, such as the operations for cancer of thoracic esophagus which necessitates bilateral opening of chest, can not be performed well without the use of the positive pressure machine. In such cases, the degree of positive pressure most suitable is a matter of great concern to us, though it is at present not yet quite settled.

I performed several series of experiments to study respiratory changes in cardiac rhythm of dogs by means of the "Cardiotachograph" devised by Matsuda[2] in order to know the patterns in cardio-pulmonary correlation when the chest was opened bilaterally. According to Matsuda and Suzuki[3] the extent of the respiratory arrhythmias is much modified not only by the frequency of the cardiac beat but also by the prevailing tone and sensibility of autonomic nervous system. From the results of such studies I tried to get some insight concerning the optimal positive pressure for the operation of chest opened bilaterlly.

Experimental

Methods

Healthy dogs, weighing 10 kg. to 13 kg. were used. As the changes of the position of the body have influence on the respiratory arrhythmias, all dogs were studied, being fixed in the supine position on a desk for ex-

TABLE

				30 min.		60 min.
No. 1	Control case	P. I.		1.22		1.20
		R. A.		0.81		0.77
No. 2	Control case	P. I.		1.74		1.80
		R. A.		1.01		1.11
			Preop.	10 min.	20 min.	30 min.
No. 3	Chest open case under 20cm. → 4cm. positive pressure	P. I.	0.80	0.88	0.68	0.65
		R. A.	0.03	0.58 (20 cm.)	0.53 (16 cm.)	0.46 (12 cm.)
No. 4	Chest open case under 4 cm. → 20 cm. positive pressure	P. I.	1.01	0.85	0.83	0.92
		R. A.	0.62	0.69 (4 cm.)	0.48 (8 cm.)	0.69 (12 cm.)
No. 5	Chest open case under 4 cm. → 20 cm. positive pressure	P. I.	1.47	1.16	1.09	0.89
		R. A.	1.29	0.92 (4cm.)	0.90 (8 cm.)	0.60 (12 cm.)
			Preop.	10 min.	30 min.	60 min.
No. 6	Chest open under positive pressure of 8 cm.	P. I.	1.39	1.15	1.15	1.17
		R. A.	0.75	0.59	0.70	0.71
No. 7	Chest open case under positive pressure of 8 cm. (cyclopan)	P. I.	0.86	0.81		0.80
		R. A.	0.39	0.52		0.49
No. 8	Chest open case under positive pressure of 16 cm.	P. I.	1.12	0.91		0.68
		R. A.	0.58	0.57		0.52
No. 9	Chest open case under positive pressure of 20 cm.	P. I.	1.28	1.31	0.71	0.48
		R. A.	0.71	0.91	0.30	0.12
No. 10	Chest open case under 20 → 28 cm. then 4 cm. → 20 cm. positive pressure	P. I.	1.31	0.63	0.46	
		R. A.	0.90	0.16 (16 cm.)	0.14 (20 cm.)	
No. 11	Chest open case 0 cm. → 12 cm. controlled respiration	P. I.	1.25	1.15	1.08	1.02
		R. A.	0.91	0.73	0.68	0.56
No. 12	Chest open case 0 cm. → 12 cm. controlled respiration	P. I.	1.11	1.01	0.99	0.98
		R. A.	0.82	0.79	0.78	0.80
No. 13	Chest open case assisted respiration	P. I.	1.08	1.11		0.88
		R. A.	0.65	0.58		0.50
No. 14	Chest open case under positive pressure of 8 cm. atropine inj.	P. I.	0.90	0.98	0.86	0.92
		R. A.	0.65	0.79	↑ Atropine 0.92	0.30
No. 15	Chest open case under positive pressure of 8 cm. atropine inj.	P. I.	1.22	1.10	0.98	0.97
		R. A.	0.89	0.69	↑ 0.32	0.28

P.I. = Pulse interval. R.A. = Respiratory arrhythmias. 4 cm. = Positive by 4cm. of water.

I

	90 min.		2 hrs.				
	1.08 0.78		1.12 0.75				
	1.65 1.04		1.80 0.95				
40 min.	50 min.	60 min.	70 min.	Post Op.			
0.68 0.41 (8 cm.)	0.58 0.46 (4 cm.)			0.66 0.20		0.73 0.43	0.81 0.48
0.44 0.41 (16 cm.)	0.57 0.34 (20 cm.)	0.99 0.75 (8 cm.)		0.54 0.33		0.57 0.38	0.84 0.27
0.58 0.39 (16 cm.)	0.54 0.35 (20 cm.)	0.63 0.35 (8 cm.)	0.73 0.52 (8 cm.)	0.35 0.12			
90 min.	Post Op,	24 hrs.	48 hrs.	3 days	5 days	7 days	14 days
1.12 0.61	1.06 0.51		0.45 0.17			1.02 0.76	1.28 0.81
	0.79 0.64	0.76 0.42					
	0.51 0.28						
	0.32 0.09						
0.37 0.03 (28 cm.)	$\left(2\frac{1}{2}\text{hrs.}\right)$	0.34 0.02 (4 cm.)	(3 hrs.)	0.40 0.02 (2 cm.)	$\left(3\frac{1}{2}\text{hrs.}\right)$		
	1.05 0.68	0.98 0.48		0.97 0.39		1.12 0.82	
	1.03 0.83			1.18 0.98			
	0.51 0.07						
	0.69 0.33				1.02 0.78	1.12 0.82	
	0.93 0.37				1.10 0.80		1.20 0.86

perimental convenience. As the cardiac rhythm showed striking changes in proportion to various factors, internal as well as external, especially by nervous influences, the dogs were anesthetized with morphine chloride 3 mg/Kg and 1.0 cc. to 1.5 cc. of 20% luminal solution in order to avoid all untoward effects and to keep all the same as much as possible. Thus the dogs became quite tranquil as in sleep and no sign of pain or protective movements were recognized in the operation of opening of chest, resection of nerves and aggression to the mediastinum. To institute positive pressure, semiclosed positive pressure machine with small dead space was used in order to avoid the accumulation of CO_2 as much as possible.

The sixth or seventh rib was resected from the sternal margin in front and to the axillary line in side and continuous positive pressure of 4 cm. to 8 cm. (H_2O) was given immediately before the chest was opened to avoid the sudden collapse of lung on the opening.

The cardiac rhythm was recorded by cardiotachograph continuously for an interval of several minutes. Because it seems rational that the degree of respiratory arrhythmia is measured by the difference of pulse interval in expiratory and inspiratory phase of respiration (Anrep[4] and Matsuda) I adopted also such method of measurement.

The duration of time to keep the chest open was settled at one and a half hours, and the observation were done at 10 min., 30 min., 1 hour, one and a half hours after the start, and immediately after the chest was closed and moreover 24 hours, 3rd, 5th, 7th, 10th and 14th day after the operation.

Results (See Table I)

As controls (No. 1, No. 2), the pulse interval and respiratory arrhythmias were observed during the interval of one and a half hours after falling in complete anesthesia, that is 30 min. after administration of morphine and luminal.

The changes of the puls interval and respiratory arrhythmias during the 2 hours were slight as seen in Table I. In 30 cases, including both control cases and experimental cases, in which morphine and luminal were administered, the pulse intervals were 1.73 sec.—0.84 sec. (av. 1.27 sec.) and the extent of respiratory arrhythmias was, except a case No. 3, 1.26 sec.—0.34 sec. (av. 0.74 sec.) (Fig. 1). The pulse interval and the extent of respiratory arrhythmias of unanesthetized normal dogs, as Sato[2] stated, were nearly 0.97 sec.—0.70 sec. (av. 0.83 sec.), 0.6 sec.—0.30 sec. (av. 0.48 sec.) respectively. The above figures, therefore, illustrates that the administration of morphine and luminal caused bradycardia with a increase in respiratory arrhythmia. Although the difference of effect of morphine and luminal upon each individual is fairly distinct,

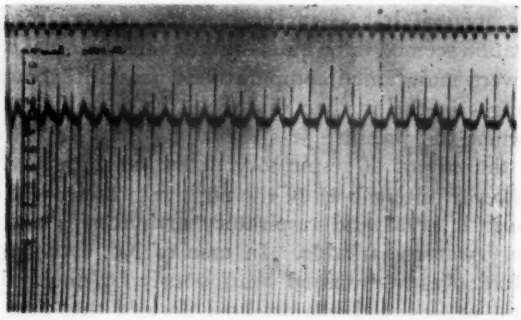

Fig. 1. Control case (30 min. after administration of morphine and luminal).

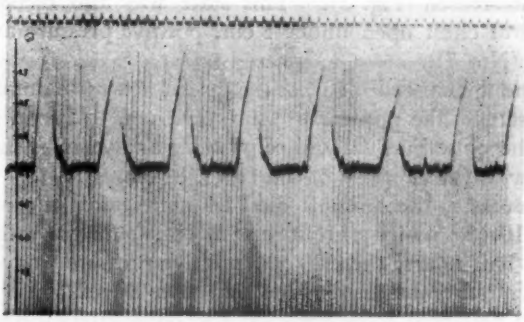

Fig. 2. Case of bilateral chest open (90 min. after operation under positive pressure of 20 cm. of water).

the changes in course of time in each case is comparatively slight as shown in control cases.

In case of No. 3 where the initial positive pressure of 20 cm. of water was lowered gradually by 4 cm. per 10 min., immediately after the bilateral opening of the chest, the respiration got very deep and slow and slight increase in the pulse interval was seen and the extent of respiratory arrhythmias increased remarkably. (Fig. 2) But in course of experiment as described above, tachycardia gradually appeared, while the fairly high pressure was still maintained. However, the sign of elongation of pulse interval reappeared, when the positive pressure was lowered to 8 cm. and was maintained for 10 min.

In No. 4 and No. 5, the positive pressure which was initially 4 cm. high, was raised gradually at a rate of 4 cm. per 10 min., then the lungs became collapsed and the struggling respiration ensued immediately after

the chest was opened, but when the positive pressure rose to 8 cm.—12 cm. the respiration seemed to be quiet and the pulse interval and respiratory arrhythmias were maintained comparatively constant, showing almost no irregular changes in the cardiac rhythm as compared with preoperative state. But when the pressure was raised to 16 cm. or 20 cm. the pulse interval and the extent of respiratory arrhythmias got remarkably decreased. However puting both of the positive pressure to 8 cm. increased again the pulse interval and respiratory arrhythmias and especially in No. 4 recovery was remarkable. In case of No. 6, where the positive pressure of 8 cm. was maintained continuously for one and a half hours while the chest was opened bilaterally, I noticed that rate and depth of respiration showed almost no change as compared with preoperative state, and the pulse interval and the extent of respiratory arrhythmias got slightly decreased (Fig. 3). This state was also recognized in case of the bilateral chest open under 8 cm. positive pressure and anesthesia of cyclopan (No. 7).

When the unchanged high positive pressure was maintained continuously, for instance, the positive pressure of 16 cm. to 20 cm. for one and a half hours, the pulse interval and respiratory arrhythmias showed striking diminution with the progress of time (No. 8, No. 9).

In these cases, the animals died 24 hours after the operation. In case of No. 10, the positive pressure of 20 cm. to 28 cm. was initially used for 2 and a half hours, then 28 cm. positive pressure was suddenly converted to 4 cm. and was further lowered after 30 min. into 2 cm. positive pressure. In this case I noticed that the pulse interval and respiratory arrhythmias under 20 cm. to 28 cm. positive pressure were very small and also exceedingly small under 4 cm. to 2 cm. positive pressure. Thus I found that there is no essential difference between the effects of extermely high or low positive pressure, such as 28 cm. or 2 cm.

When the controlled respiration, that is fluctuation of the positive pressure from 0 cm. to 12 cm, was carried out 14 to 16 times in one minute the dog's respiration was calm in accordance with this procedure. In these cases (No. 11, No. 12), both the pulse interval and respiratory arrhythmias showed but slight changes.

No. 11 was a case in which respiratory paralysis occured immediately after the chest was opened, but was revived by means of controlled respiration instead at once. But it is worthwhile to call attention to the fact that the distinct changes in the pulse interval and respiratory arrhythmias were not recognized during the controlled respiration under the state of the respiratory paralysis as compared with preoperative state (Fig. 4).

In the assisted respiration during the bilateral chest open, the pulse

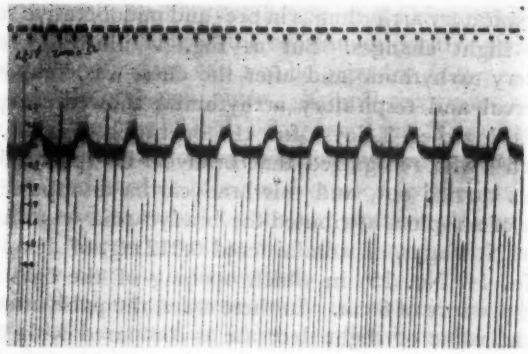

Fig. 3. Case of bilateral chest open under positive pressure of 8 cm. of water (30 min. after operation).

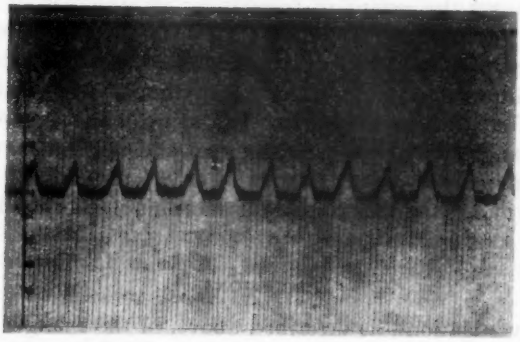

Fig. 4. Case of controlled respiration (30 min. after operation).

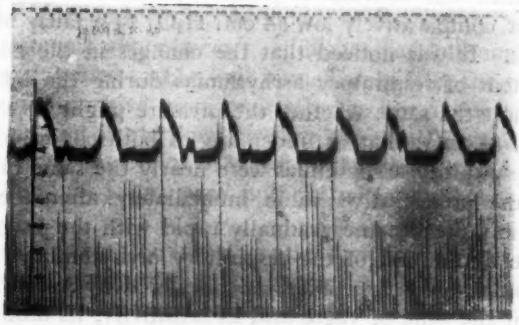

Fig. 5. Case of assisted respiration (90 min. after operation).

interval and respiratory arrhythmias in pre- and midoperative state showed comparatively slight changes. But as Fig. V illustrates, the cardiac rhythm was very arrhythmic and after the chest was completaly closed the pulse interval and respiratory arrhythmias showed conspicuous decrease and this dog died 3 days after the operation (Fig. 5).

Sauerbruch[6] who recognized that bradycardia appeared, when the chest open was carried out, and this bradycardia disappeared after the administration of atropine, attributed the bradycardia was due to the increased tone of N. Vagus. I administered 0.012 mg. of atropine per Kg. in 2 cases (No. 14, No. 15) in which opening of the chest was done under positive pressure of 8 cm. In these cases, the pulse interval showed almost the same value or slight decrease as compared with preoperative value, but the respiratory arrhythmia got decreased strikingly.

Concerning to the postoperative course of the experimental cases, in which the bilateral chest open was carried out, I acknowledged the pulse interval and the extent of respiratory arrhythmias 24 hours after the operation were almost equal or more decreased to those when the chest was closed and the degree of recovery afterwards had a close relation to the pulse interval and respiratory arrhythmias when the chest was closed, and after all proportional to the extent of the aggression.

And it was clear that the pulse interval and respiratory arrhythmias went back to preoperative value about 14 days later and the bradycardia characteristic to the administration of morphine chloride and luminal reappeared.

Discussions

In the investigations, as described above, of the changes in the pulse interval and the extent of respiratory arrhythmias, I observed the influences which the operation of bilateral opening of the chest gives to the living body under the continuous positive pressure of various degrees ranging from a comparatively low (4 cm. H_2O) to a fairly high (20 cm. H_2O) pressures. It was noticed that the changes in the pulse interval and in the extent of respiratory arrhythmias during the bilateral chest open were almost the same whether the pressure might be set high (16-20 cm.) or low (4 cm.); that is, under any of both conditions, the pulse interval and respiratory arrhythmias were nearly the same or a little bit smaller than the preoperative value immediately after the chest was opened, but the pulse became gradually rapid with the progress of time along with the diminution of the respiratory arrhythmias.

As the respiratory arrhythmias, as considered by Anrep and Matsuda, depends on the tone of vagus and its sensitivity, its diminution with concomitant tachycardia can be regarded as due to depression of the vagal

tone, this is obviously an abnormal state of a living organism.

From such a point of view, the continuous positive pressure of 16 cm. to 20 cm. or 4 cm. of water can not be regarded as the optimal positive pressure for the bilateral chest open. Kahn[7] recognized that the blood pressure descended in case of high positive pressure Yugaya[8] & Ishihara[9] established by experiments on rabbits that when the chest was opened both respiratory and cardiac rate got decreased by the institution of high positive pressure and increased by the low positive pressure. They concluded from these observations that too high or low positive pressure would cause evil influences upon a living body.

Ishikawa[10] obtained the same results in rabbits. In my experiments on dogs, there were 6 cases in which the bradycardia got still marked immediately after the chest was opened than in the preoperative state. I would especially call attention to the fact that the pulse got more retarded than that in the preoperative state, where it was already distinctly bradycardic by the administration of morphine and luminal; that is, it indicated hypertonic state of N. Vagus.

There was, however, noticed in almost all cases tachycardia in high or low positive pressure.

In case of No. 10, in which I intended to examine the difference in effects between extremely high or low positive pressures, such as 28 cm. or 2 cm. positive pressure, I ascertained that under 28 cm. or 2 cm. positive pressure, the pulse interval and the extent of respiratory arrhythmias were exceedingly decreased and further experienced no sign of bradycardia owing to the accumulation of CO_2 and deficiency of O_2, as often seen in experiments on rabbits.

It seems to me that this fact is due to the difference in the reaction pattern of the tone of N. Vagus between both species of animals. While the rabbits show tachycardia and very weak respiratory arrhythmias and exhibit often bradycardia, when one of their vagal nerves was resected at its root, the dogs have quite the opposite disposition.

The experiments above seem to indicate that, although the tone of N. Vogus is maintained rather well immediately after the bilateral chest open whether the positive pressure is high or low, it decreases with the progress of time and in the long run cardiac weakness ensues.

In view of the cases No. 6 & No. 7, I acknowledged that positive pressure of 8 cm. to 12 cm. was optimal when the chest was opened bilaterally.

Operations, such as the intrathoracic vagotomy and thoracic esophagectomy, were all carried out under continuous positive pressure of 8 cm. and it was established that this positive pressure, caused little influences on the living body.

Ishikawa examined the cardiac output and E.C.G. of the rabbit during the bilateral chest open and pointed out the continous positive pressure of 8 cm. was the optimal condition.

Saundress[11] also recognized that 4 cm. to 8 cm. positive pressure was necessary during the bilateral chest open in considering the clinical findings. My experiment also showed the same results as theirs.

Two cases in which controlled respiration was done, no remarkable change were seen in the pulse interval and respiratory arrhythmias both before and during the operation and the course after the operation was smooth in a similar manner as in the case of the positive pressure of 8 cm. This fact is in accordance with what Moresh[12] has pointed out.

In case No. 11, where the dog fell into respiratory paralysis soon after the chest was opened, because of the failure of the connection to the positive pressure machine, the respiratory arrhythmias were recognized to occur in accordance with inflation of lungs, when the controlled respriration was carried out. Konell,[13] H. E. Hering[14] & Anrep put stress on the peripheral theory of respiratory arrhythmias: these are the result of a reflex which originating in the lung, reaches the centrum through the sinsible fibres of N. Vagus and acts centrifugally on the heart. The result of my experiment is quite in accord with this theory.

Crafford's[15] experiments showed the assisted rerespiration is effective againest the accumulation of CO_2, but my experiment illustrates that it is technically difficult to do the assisted rspiration keeping pace with the respiratory rhythm experimental animal; cardiac rhythm often becomes irregular and the vagal nerves soon fall into fatigue.

More-over, when atropine was administered during the bilateral chest open under the most adequate positive pressure (8 cm), that is when the pressure was neither too high nor too low, the pulse interval showed almost no changes and the respiratory arrhythmias got remarkably diminished. That is, the excessive increase in tone of vagal nerves, as Suerbruch stated, was not recognized with the progression of time after the chest was opened.

Conclusions

It is not infrequent that the bilateral chest open is obligatory in the operation of thoracic esophagus. And it goes without saying that the positive pressure machine is absolutely necessary in order to perform the operation safely during the chest open.

I examined the changes in cardiac rate of dogs by means of "Cardiotachograph (A type)" devised by Matsuda, aiming to study the influences which various sorts of continuous positive pressure (seimclosed system) would exercise upon the living body.

This experiment revealed that the positive pressure of 8 cm. to 12 cm. of water caused the slightest changes both in the pulse interval and respiratory arrhythmias, as compared with preoperative value.

And when the positive pressure became higher or lower than the range from 8 cm. to 12 cm, the pulse interval and respiratory arrhythmias decreased markedly except for a comparatively short time immediately after the chest was opened.

Such changes seem to correspond fairly well to the suffering of the living body.

While some cases showed the increase in tone of N. Vagus with bradycardia and increase of respiratory arrhythmia immediately after the chest was opened, almost all cases showed tachycardia and diminution of respiratory arrhythmias during the bilateral chest open.

It took almost 14 days for the changes in cardiac rhythm by the bilateral chest open to be extinguished, the recovery of cardiac rhythm ran keeping pace quite well with the recovery of the general condition of animals.

With controlled respiration under the positive pressure from 0 cm. to 12 cm., there occurred very slight changes in cardiac rhythm.

This fact, besides the clinical experiences reported thus far, indicates that the controlled respiration should be used.

Assisted respiration was found not adequate, as it is a very difficult procedure.

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Studies on the Cardiac Rhythm of the Dog during Bilateral Thoracotomy and after Vagotomy and Esophagectomy

II. On the Cardiac Phythm of the Dog in the Cervical and Intrathoracic Vagotomy

By

Wataru Kojima
(兒島 渡)

(From the Surgical Clinic of Prof. S.-T. Katsura, Faculty of Medicine, Tohoku University. Sendai)

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Introduction

It is not uncommon that in the operation of esophagus particulary of the radical operation of cancer of esophagus, purposely or by accidence, the vagus trunk and some of its branches are cut or damaged in cervical region or in thorax, especially when carcinomatous infiltration has invaded into mediastinum or tissues around the nerve.

In the previous communication about the cardiotachographic examinations on dogs during the bilateral chest open, it was reported that aggression given to the animals caused the changes in the pulse interval and respiratory arhythmias directly or indirectly through vagal nerves, and the degree of these changes brought about by changes in tone of N. Vagus indicated in a sense the extent of aggression.

Here, in this second paper, I will report the observation on the changes in the pulse interval and respiratory arhythmias, when the vagotomy was performed on one side or both in cervical or in thoracic region.

While it is already known by Anrep's[1] experiments that the respiratory arhythmias disappear almost completely by the complete resection of branchial rami of N. Vagus, I aimed to study experimentally what influences may be exercised upon the animals when a trunk of N. Vagus is cut in various regions.

The method of study is to observe changes in cardiac rhythm, respiratory arhythmias in particular, by means of cardiotachograph as in the previous report.

Experimental

Methods

Normal dogs, weighing 10 kg. to 13 kg., to which morphine cholride and luminal were administered in a similar manner as in the foregoing experiments, were used. In order to avoid shock as much as possible, which is often caused by damage or resection of nerve, the operation was not carried out, until it was clear that experimental animals were completely anesthetized, and further local anesthesia by 0.05% percamin was given around and to nerve trunk itself.

In some experiments, the vagotomy in cervical region was done at the height of the middle between cartilage thyreoidea and clavicle.

In other experiments the vagotomy was carried out in thorax, opening of the chest was maintained under the continuous positive pressure of 8 cm (of water). When the vagal amputation was carried out above the bifurcation of bronchus, the incision of about 2 cm. long in the mediastinum at the portion of 3 cm. above the bronchial bifurcation and the nerve trunk only was resected on right or left or on both sides.

In the case in which the vagotomy was carried out on both sides below the bronchial bifurcation, not only nerve trunk but also all of its branches were resected.

To observe the immediate influences of the operation, I examined particularly the pulse interval and respiratory arhythmias 10 min., 30 min., 1 hour, later, but the changes of them in later stages such as 24 hours, 3rd, 5th, and 7th day after the operation were also studied.

Results (See Table I)

In addition to the resection of the stem of N. Vagus, carried out by the above mentioned method in the cervical region, the 2nd and 3rd branches of N. cardiacus and N. recurrens were also cut.

When the vagotomy was first done on the right side (No. 16) the pulse interval and respiratory arhythmias showed remarkable decrease about 30 min. after the operation and diminution of respiratory arhythmias was conspicuous; but slight recovery was seen 1 hour after the operation.

In No. 17 the changes in the pulse interval and the extent of respiratory arhythmia were conspicuous immediately after operation and, 1 hour after the operation these values were 0.48 sec. and 0.02 sec; this is a striking decrease as compared with preoperative value. Thereafter the respiratory arhythmias disappeared almost completely. The dog of this case died 20 hours after the operation without recovering from narcosis.

And in two cases (No. 18, No. 19) where the vagotomy was done on the left cervical region, the pulse interval and respiratory arhythmia got

TABLE I

			Pre-Op.	Post-Op. 10min.	30 min.	60 min.	90 min.	24 hrs	48 hrs	3 days	5 days	7 days	10 days	14 days
No. 16	Vagotomy right side in cervical region	P. I.	1.42	1.02	0.82	0.98		1.01				1.52		
		R. A.	0.94	0.46	0.22	0.42		0.51				1.04		
No. 17	Vagotomy right side in cervical region	P. I.	1.25	0.54		0.40	0.50							
		R. A.	0.68	0.20		0.02	0.03							
No. 18	Vagotomy teft side in cervical region	P. I.	1.49	0.62	0.65	0.36					0.69		1.38	
		R. A.	1.09	0.29	0.28	0.08					0.37		0.99	
No. 19	Vagotomy left side in cervical region	P. I.	1.31	0.64	0.51	0.33	0.34							
		R. A.	0.72	0.31	0.49	0.01	0.01							
No. 20	Vagotomy right side above bronchial bifurcation	P. I.	1.61	0.83	0.84	0.87	0.98	1.02		1.10	1.60		1.45	
		R. A.	0.81	0.40	0.41	0.47	0.51	0.53		0.71	1.09		0.78	
No. 21	Vagotomy left side above bronchial bifurcation	P. I.	1.36	1.15	1.29	0.77	0.79	0.81		1.08				
		R. A.	0.13	0.51	0.54	0.35	0.41	0.48		0.69				
No. 22	Vagotomy both side above bronchial bifurcation	P. I.	1.70		1.35	1.07	1.05		1.30					
		R. A.	0.90		0.57	0.35	0.37		0.71					
No. 23	Vagotomy both side below bronchial bifurcation	P. I.	1.43	1.57	1.44	1.29		1.30					1.50	
		R. A.	0.67	0.79	0.68	0.57		0.71					0.80	

P.I=Pulse interval. R.A.=Respiratory arrhythmias.

remarkably decreased and further 1 hour later these values revealed 0.36 sec. and 0.08 sec. (in No. 18); 0.34 sec. and 0.01 sec. (in No. 19) respectively, that is surprising decrease as compared with preoperative value (Fig. 1).

The dog of No. 19, died 10 hours after the operation in an unconsicous condition.

The vagotomy on the cervical region caused very little changes in the depth and rate of respiration. In cases in which the vagal amputation was done on the right or left side above the bronchial bifurcation, the pulse interval and respiratory arhythmia got moderately decreased within one hour after the operation (No. 20, No. 21).

and in case (No. 22) in which vagal amputation was done on both sides above the bifurcation, the pulse interval and respiratory arhythmias showed moderate decrease in similar manner as in the experiments just above described (Fig. 2).

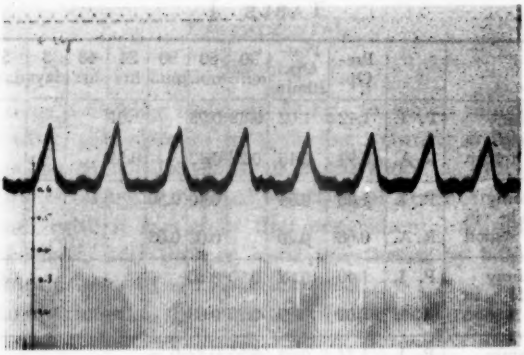

Fig. 1. Vagal amputation on the left side in cervical region (60 min. after operation).

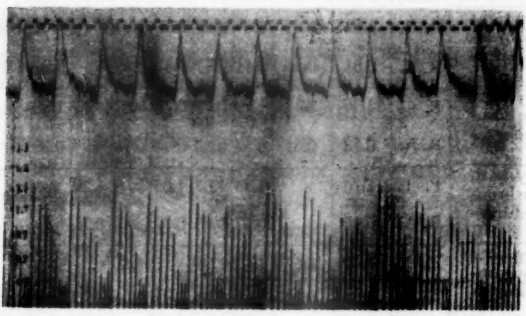

Fig. 2. Vagal amputation on both sides above bronchial bifurcation (90 min. after operation).

In case (No. 23) of the vagal amputation below the bronchial bifurcation, the changes in the pulse interval and respiratory arhythmias were unexpectedly slight.

In the surviving cases, postoperative recovery progressed gradually to become complete in about 10 days after the operation.

This process is closely related to the degree of changes in the cardiac rhythm when the vagotomy was done.

Discussion

The vagal nerves play a very important rôle in autonomic nervous system, wich governs the vegetative functions of a living body. Numerous reports on the functions of N. Vagus have been so far published, particulary E.R. Woodward,[2] Feldman,[3] Crimson,[4] F. Reichsman[5] and others studied the effects of the amputation of N. Vagus in various regions

upon the living body. But almost all investigations aimed to study the effects of vagotomy upon the digestive tract or upon the pulmonary edema, and Dragstedt[6] and Walter's report on the therapeutic effects of vagotmy just above or below diaphragma for gastric ulcer and chronic ulcerative colitis is well known.

However, there is not yet complete agreement in opinion as to whether the vagotomy in the cervical region or above the bronochial bifurcation is compatible with life or not. Some authers as Garlock and Nissen[7] emphasized the influences which vagal amputation above the bifurcation may exhibit upon the living body expressing the influences as "Vagus disturbances," but other authors deny it. In my experiment, striking changes in the pulse interval and respiratory arhythmias were noticed when cervical vagotomy, not excluding the second and third branches of N. cardiacus, was done, and despite of very small operative aggression, the degree of the above mentioned change was surprisingly great.

And in each case in which the vagotomy was done on the right or left side, there followed the almost complete disappearance of the respiratory arhythmias and animals died.

When vagal amputation was done on the left side in cervical region only, the changes in the pulse interval and respiratory arhythmias seemed to be more remarkable than on the right side both in serviving as well as in dead case.

How should be these changes in the pulse interval and respiratory arhythmias interpreted?

The remarkable changes in the pulse interval and respiratory arhythmias observed in the experiments of the present communication are not to be considered as caused by neurogenic shock, for these findings are signs of decreased vagal tone and the animals were sufficiently anesthetized throughout the experiments. When the function of the cut vagus was compensated by the remaining vagal nerve on the other side, the respiratory arhythmias were still noticed, though much decreased. In such cases the animal survived the operation. On the other hand, in cases where the respiratory arhythmias disappeared almost completely, the animals died at length.

Differences in the attitude of animals mentioned above seem to be due to individual difference in disposition. When the amputation of N. Vagus above the bifurcation, the amputation of N. cardiacus could be avoided. In this type of experiment, not withstanding the aggression was exaggerated as the chest was opened in addition to vagotomy, the changes in the pulse interval and respiratory arhythmias were slight as compared with the changes on cervical vagotomy, and there was no essential difference in effects between right and left and on both sides; the

general influences upon the animals were slight. Considering these results, I do not think that the vagotomy itself above the bifurcation does not cause any serious effects on life as it is generally considered.

More-over, when the vagotomy on both sides below the bifurcation was done, the pulse interval and respiratory arhythmias showed almost no changes between pre- and postoperative value.

This results seems to indicate the fact that the vagal amputation directly above diaphragma causes only slight influences on the function of the living body.

In all cases in which vagotomy was carried out, the changes in the pulse interval and respiratory arrhythmias were most remarkable about 1 hour later and the changes at this time seemed to me to be much related to prognosis.

And the lower the region of vagal amputation from the cervical to that above and below the bifurcation, the less became the degree of the changes in cardiac rhythm and less the dangers to the living body.

Such facts, as described above, indicate that the amputation of N. cardiacus, even if it is not total, is often fatal.

Conclusions

I amputated N. Vagus on the cervical region, above and below the bifurcation of bronchus in thorax and examined the changes in the cardiac rhythm compared with the extent of aggression to the living body. When the vagal amputation was done in the cervical region including the large branches of N. cardiacus, the pulse interval and the extent of respiratory arhythmias revealed surprising decrease, whether on the right or the left side.

Especially it is noteworthy that 2 cases where one sided vagotomy in cervical region was performed, showed the almost complete disappearance of respiratory arhythmias, and died at the end of 10 hours course of narcosis following the operation.

And when vagal amputation was carried out above or below the bronchial bifurcation on each side or both in thorax, there was noticed some difference in the cardiac rhythm between above and below, the changes in cases above being more marked than in case below.

However, despite of increased aggression, such as the chest open, the changes of the cardiac rhythm as well as of the general condition of animals were unexpectedly slight.

The changes in cardiac rhythm described in detail in this part as a sin of removal of one vagus nerve, subsided gradually in from 3 days to about 10 days after the operation. Such a spontaneous recovery to

normal condition seems to be due to functional compensation of N. Vagus on the contralateral side.

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Studies on the Cardiac Rhythm of the Dog during Bilateral Thoracotomy and after Vagotomy and Esophagectomy

III. On the Cardiac Rhythm when Total Exstirpation of Thoracic Esphagus is Performed

By

Wataru Kojima
(兒島 渡)

(From the Surgical Clinic of Prof. S.-T. Katsura, Faculty of Medicine, Tohoku University, Sendai)

(Received for publication, December 16, 1951)

Introduction

There are two methods today for the radical esophagectomy followed by cervical esophagogastrostomy. The one is the intrathoracic gastric transplantation, as Sweet,[1] Garlock[2] and many other authors performed, the other is Nakayama[3]s[3] antithoracic esophagogastrostomy.

A decision can not be made hastily as to which one of the foregoing two methods is superior. However, there is no fundamental difference between the two method in that both perform the total exstirpation of thoracic esophagus. The only difference consists chiefly in the manner of esophagogastrostomy.

And concerning the question what influences would be exercised by aggression on mediastinum containing the great vessels and important nerve stems, there is still diversity of opinions.

Following my studies on the cardiac rhythm during the chest open or after the amputation of N. Vagus as described in Part I and Part II, I aimed to investigate the cardiac rhythm of dogs, when radical esophagectomy and cervical esophatogastrostomy was experimentally performed, for elucidation of the influences, which this procedure would cause to a living body.

Experimental

Methods

Healthy dogs, weighing 10 Kg to 13 Kg, were anesthetized with morphine and luminal in a similar manner as in foregoing experiments.

In No. 24, and No. 25, the operation was performed by Sweet's method. In No. 26, and No. 27 Nakayama's method i.e. radical thoracic esophagectomy with antithoracic gastric implant was adopted.

An incision was made in the upper abdomen and the stomach was completely mobilized from the surrounding tissues. After it was acertained that the stomach could be carried to the cervical region, the abdominal wound was closed.

Then thorax was opened on the right side under the continous positive pressure of 8 cm. (of water) and esophagus was freed from mediastinum. While, in Sweet's method, the stomach was elevated through the right thorax and right cervical esophagogastrostomy was performed, in Nakayama's method, the stomach was elevated through a skintube in front of the sternum and the anastomosis with the stump of esophagus was made.

In No. 28, and No. 29, only thoracic esophagectomy was carried out and esophageal fistula was made in cervical region. As a control, subtotal gastrectomy was also performed in No. 30.

Results

In two cases, in which Sweet's method was used, the pulse interval and the extent of respiratory arhythmias were respectively 0.48 sec, 0.16 sec. (in No. 24); 0.41 sec. 0.01 sec (in No. 25), about one hour after the operation. Remarkable decrease of both was to be noted, in No. 25, respiratory arhythmias disappeared almost completely (Fig. 1). In two cases, in which Nakayama's method was adopted, the pulse interval and respiratory arhythmias were each 0.60 sec, 0.03 sec (in No. 26); 0.32 sec, 0.01 sec (in No. 27). These show a striking diminution (Fig. 2).

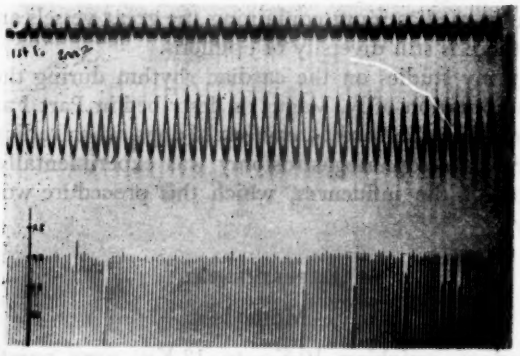

Fig. 1. Case of radical esophagectomy with intrathoracic gastric inplant (Sweet's Method).

TABLE I

			Pre-op.	Post-op. 10min.	30 min.	60 min.	90 min.	24 hrs.	48 hrs.	3 days	5 days	7 days
No. 24	Radical esophagectomy with intrathoracic gastric implantation	P. I.	1.46		0.50	0.48			0.43			
		R. A.	0.85		0.19	0.16			0.12			
No. 25	Radical esophagectomy with intrathoracic gastric implantation	P. I.	1.52	0.42	0.41		0.57	0.54				
		R. A.	0.91	0.03	0.01		0.01	0.01				
No. 26	Radical esophagectomy with antithoracic gastric implantation	P. I.	0.92	0.63	0.60	0.63	0.56					
		R. A.	0.67	0.05	0.03	0.04	0.05					
No. 27	Radical esophagectomy with antithoracic gastric implantation	P. I.	1.27	0.54		0.32	0.42					
		R. A.	0.46	0.02		0.01	0.01					
No. 28	Radical esophagectomy with esophageal fistula	P. I.	1.32	0.74		0.65		0.54				
		R. A.	0.72	0.06		0.09		0.23				
No. 29	Radical esophagectomy with esophageal fistula	P. I.	0.80	0.39		0.52	0.65		0.61			
		R. A.	0.30	0.14		0.10	0.17		0.09			
No. 30	Subtotal gastrectomy	P. I.	1.49	1.11	1.04		1.05			1.25		
		R. A.	0.95	0.73	0.64		0.64			0.79		

P.I.=Pulse interval. R.A.=Respiratory arrhythmias.

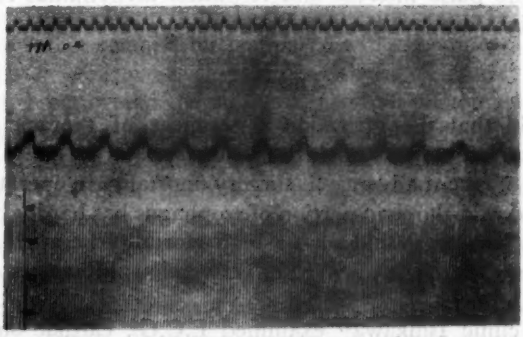

Fig. 2. Case of radical esophagectomy with antithoracic gastric implant (Nakayama's method).

Both experimental series showed conspicuous diminution of respiratory arhythmias. Particulary in No. 26, the respiratory arrhythmias decreased more remarkably than the the pulse interval. Further the disappearance of the respiratory arhythmias in No. 25 and No. 27 foretold

Fig. 3. Case of radical esophagectomy with only esophageal fistula.

an evil prognosis as both cases died within 24 hours after the operation.

In No. 28 and No. 29, where the aggression was limited to only the toal resection of thoracic esophagus, the pulse interval and respiratory arrhythimas got remarkably decreased (Fig. 3).

Though the decrease in these cases were less than in foregoing experiments, the changes seem to be too great compared with the extent of aggression.

In the experimental case, in which subtotal resection of stomach, an aggression almost equal in degree to the radical esophagectomy, was performed, the pulse interval and respirator arrhythmias revealed comparatively slight diminution.

Discussions

It is said that the operation for cancer of thoracic esophagus is the last fence left to be attacked in surgery.

In spite of recent advance in surgery, mortality in operation of cancer in upper- and mid-thoracic portion of esophagus has hardly been lowered.

To what causes, should such a high mortality be attributed.

Of course, the chest open gives very serious influences on heart and lungs.

In our clinic Ishikawa[4] examined E.C.G., cardiac output and the degree of oxygen saturation of blood, he agreed in that the chest open under 8 cm. positive pressure caused the least influences on experimental animals.

My experiment, as already stated, revealed likewise that optimal positive pressure can remove one of causes of death in the radical esophagectomy with esophagogastrostomy.

Now the second problem; does the aggression to the mediastinum give serious disturbance to a living body or not?

I should like to insist on the great influences of the aggression on the mediastinum, which comprises large vessels and important nerves.

As reported above, no distinct difference in the pulse interval and respiratory arhythmias between 2 cases of Sweet's method and 2 cases of Nakayama's method.

Although I performed the chest open under optimal positive pressure of 8 cm. and it was sure that I did not injure stem of N. vagus, the pulse interval and respiratory arhythmias showed remarkable decrease postoperatively and in No. 25 and No. 27, respiratory arhythmias almost completely disappeared, in No. 24 and No. 26 the respiratory arhythmias were so slight that it could be hardly measured.

It is worthy of attention that these cases are very similar in sign to dead cases in the vagal amputation on the cervical region.

And the prognosis of the foregoing 2 cases (No. 25, No. 27) were absolutely evil. Can we conclude that this cardiac rhythm indicating the almost complete disappearence of vagus tone is caused by the aggression on mediastinum?

As the mobilization of stomach throughout and its elevation into the cervical region are not a negligible aggression, I performed the operation of esophagectomy only, the operation almost limited to mediastinum and thoracic esophagus.

This experiment showed that the changes observed in the pulse interval and respiratory arhythmias were somewhat less than in foregoing cases, but were rather remarkable compared with the appearant slightness of aggression.

Therefore, I performed the subtotal resection of stomach as control, but the changes in the pulse interval and respiratory arhythmias were unexpectedly slight in pre-and post-operative state.

This facts, that even the simple mobilization and exstirpation of thoracic esophagus exercises nearly the same influence on the cardiac rhythm as the radical esophagectomy with cervical esophagogastrostomy and there was comparatively slight influence by subtotal gastric resection, convinced me that importance of aggression to mediastinum can not be denied.

And the results that cardiac rhythm is nearly the same as in cases of vagal amputation in cervical region, is easily understood if man think that N. cardiacus and plexus cardiacus superficialis et profundus are prone to be injured by manipulation near the aortic arch or around upper and mid thoracic portion of esophagus.

The clinical facts that the postoperative courses are smooth when

aggression to mediastinum is very small, such as in the operation of esophageal diverticle and of lower portion of esophagus, are quite in accord with the results of my experiments reported above.

Conclusions

I performed the total exstirpation of thoracic esophagus with esophagogastrostomy on dogs, and observed the changes in the pulse interval and respiratory arhythmias due to this procedure.

There was noticed remarkable decrease in these quantities both in Sweet's and Nakayama's methods of operations.

In cases where the disappearance of respiratory arhythmias was so maked as in the vagal amputation on the cervical region, prognosis was unfavorable.

These results suggest, that the damage and injuries to N. cardiacus in the aortic area or plexus cardiacus around the upper and mid thoracic portion of esophagus are important causes of high mortality in the radical operation of thoracic esophagus.

I desire to express my most sincere appreciation to Prof. Matsuda for his constant advice and encouragement throughout my these experiments.

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Studies on Malignancy of Goiters

By

Yoshio Ito
(伊藤 順夫)

(From the Surgical Clinic of Prof. S.-T. Katsura, Faculty of Medicine, Tohoku University, Sendai)

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In spite of the extreme rarity of autopsy cases, thyroid cancer has been experienced not infrequently in our clinic. The purpose of this paper is a revision of nodular goiters in a wider sense from clinico-pathological aspects, trying to establish a new classification of struma maligna. In this connection, blood vessel and capsule invasion (Graham[1)2)]) were examined carefully.

Material and Methods

Histologic specimens and clinical histories of 185 cases of nodular goiters, observed in our clinic during the past 14 years, were examined. Excluding the cases, to whom surgical approach was avoided, there were clinically 161 cases of benign goiters, 18 cases of malignant goiters and 6 cases of suspected malignancy. Most of the histologic specimen were newly prepared, although there were several cases in which only former slides were available. By means of large paraffin sections, various parts of the peripheral zone of the tumor were intended for examination. Besides ordinary staining methods, Weigert's elastica staining and silver impregnation by Bielshowsky-Foot were performed. To a letter of inquiry about postoperative fates, 97 answeres were given (cases within $\frac{1}{2}$ year after operation were excluded).

Results

1) 18 cases diagnosed clinically as malignant included 11 cases of adenocarcinoma, 6 cases of papillary carcinoma and 1 case of carcinoma simplex histologically. 4 cases of adenocarcinoma were complicated by bone metastasis (cranium, sternum, pelvis and femur[3)4)5)]). On 3 other cases, there were metastases in the cervical lymphnodes, I case presenting a pulmonary metastasis. Furthermore, 1 case (female aged 31) presented a cranial metastasis $10\frac{1}{2}$ years after operation. All 6 cases of papillary carcinoma were complicated by metastases in the cervical lymphnodes,

although their microscopic picture seemingly looked like a benign adenoma.

1 case of carcinoma simplex with metastasis in the cervical lymphnodes recurred ½ year after operation, presenting a pulmonary metastasis. Excluding 2 cases on which only the bone metastasis was examined histologically, there were marked " capsule invasions " (CSI) in all cases, and in a lesser degree (9 cases) " blood vessel invasions " (BVI) were also discernible.

2) 6 cases, clinically suspected of malignancy, included 5 cases of paillary carcinoma and 2 cases of papillary cystadenoma histologically. There were marked CSI in the former, while the latter lacked these entirely. There was, however, no marked difference on the tumor cell itself.

3) Out of 161 cases diagnosed previously as a benign adenoma, 1 case of carcinoma simplex was detected at first. Owing to a long survival (14 years) after operation, it has been regarded as a doubtful case clinically, in spite of a huge cellular tumor.

Histologic examination revealed, however, a malignant nature from a positive finding of BVI and CSI. The lower degree of them would be due to successful operation. 10 cases were classified as papillary carcinoma on the viewpoint of marked CSI, while there was no positive finding of it on 3 cases of papillary cystadenoma and 2 cases of parencymatous adenoma. Excluding these 16 from 161 cases, there remained 42 cases of toxic nodular goiter and 103 cases of non-toxic nodular goiter. Among these 145 cases of nodular goiter, 1 case of recurrence and 1 case of death by jaundice were noticed by a inquiry. There was, however, neither a case of a malignant change supposable, nor a positive finding of CSI and BVI on the histologic picture.

4) By means of a searching inquiry into postoperative fates, death by recurrent tumor was noticed on both groups of papillary cystadenoma and parenchymatous adenoma. These groups should, therefore, be classed in a precancerous thyroid adenoma for the sake of clinical convenience.

5) Summarizing the foregoing data, malignant goiters of our material were classified as follows:

Papillary Carcinoma	20
Adenocarcinoma	11
Carcinoma simplex	2
Precancerous thyroid adenoma	7
(Papillary cystadenoma	5
Parenchymatous adenoma	2)
	40

6) 40 cases of the malignant goiter are arranged on table 1 according to ages. Various factors will be observed on this table.

Fig. 1

Fig. 2

Fig. 1. Papillary carcinoma invading a large blood vessel (Case 20, low power view).
Fig. 2. BVI of a metastasizing goiter (Case 23, low power view).

Fig. 3

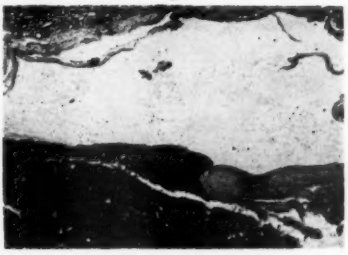

Fig. 4

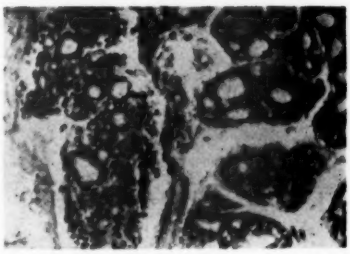

Fig. 3. Adenocarcinoma presenting marked BVI (Case 21, low power view).
Fig. 4. BVI in adenocarcinoma (Case 27, high power view).

Fig. 5

Fig. 6

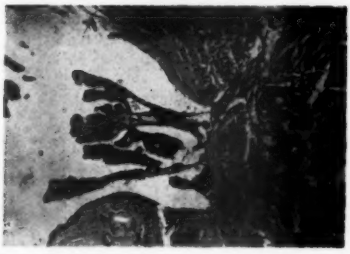

Fig. 5. Tumor-cells floating in a capillary (Case 21, high power view).
Fig. 6. Papillary carcinoma invading a thick capsule (Case 16, low power view).

Fig. 7

Fig. 8

Fig. 7. CSI in a papillary carcinoma (Case 10, low power view).
Fig. 8. Ibid. (Case 11, low power view).

Fig. 9

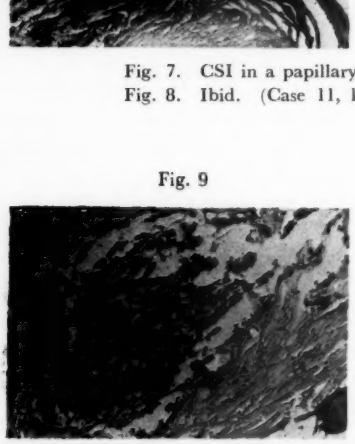

Fig. 10

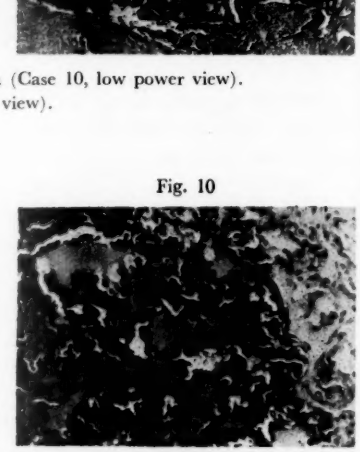

Fig. 9. CSI of metastasizing goiter (Case 23, high power view).
Fig. 10. Adenocarcinoma presenting an irregular papillary growth at primary operation (Case 22, high power view).

Fig. 11

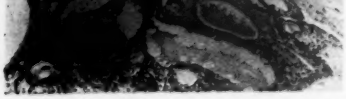

Fig. 12

Fig. 11. Structural variation at secondary operation—a picture of colloid containing vesicular adenocarcinoma (Case 22).
Fig. 12. Metastatic transformation of carcinoma simplex at cervical lymphnode (squamous carcinomalike appearance) (Case 32).

a) Age incidence: The age distribution curve as a whole presented a M-shaped figure having two peaks one at the 30 and one at the 50 decade. For the papillary carcinoma alone, there was a peak at the 30 decade; for the adenocarcinoma alone, at the 50 decade.

b) Sex: There was a deceided excess of females over males, e.g. 33:7, preseting a higher ratio than other reports.

c) Previous goiter: In the majority of cases, pre-existing struma was noticed. The longest interval ranged from 20 years for papillary carcinoma, and 17 years for adenocarcinoma. The period of a marked enlargement ranges from 1 week to 12 years.

d) Clinical symptoms: Toxic symptoms were noted only in 8 cases, of which 3 cases presented a Basedow-sign (adenocarcinoma 2, papillary carcinoma 1). Local pain was noticed in 4 cases (adenocarcinoma 1, papillary carcinoma 3). None of other symptoms, e.g. pressure feelings of the neck, dysphonia, dyspnea and loss of weight, was representative, and 11 cases were entirely symptomless. Even in 2 cases of metastasizing goiter, only bone metastasis was a prominent finding. On table 1, any clinical symptoms were marked by (+).

e) Mode of operations: Enucleation (ENC) of the tumor was performed on 14 cases; one-sided lobectomy (LED) on 11 cases; partial thyroidectomy (PTE) on 9 cases; subtotal thyroidectomy (STE) on 3 cases. 1 case of papillary cystadenoma (No. 35) was inoperable, although 5 years elapsed until death. Primary tumor of 1 case of metastasizing goiter (No. 25) was removed at another hospital. One other case (No. 20) was not suspected of a thyroid cancer at the time of operation, as there was no struma except of a prominent head tumor, of which histologic examination revealed a metastatic thyroid cancer, thus lacking a primary tumor.

f) Affected lobe: The left lobe was affected more frequently than the right (L 18: R 14), although 8 cases were difficult to judge. 1 case (No. 52) was regarded as a tumefaction of the aberrant lateral lobe.

g) Multiplicity: In the majority of cases, single nodule was found microscopically; 6 cases multinodular and 5 cases difficult to judge. In no case, however, was multicentric cancer development decided by microscopic examination.

h) Adhesion: Papillary carcinoma presented a marked tendency of adhesion comparing with adenocarcinoma.

i) Cyst: About one-half of the cases presented a complication of cyst or a cystic degeneration. One case (No. 20) revealed a penetration of the vascular wall by tumor cells causing a hemorrhagic cyst.

j) Structural variations: There were marked structural variations in the majority of cases, especially to the adenocarcinoma, i.e. papillary

or microfollicular transformation, well differentiated colloid vesicular structure, anaplastic alveolar structure associated with intermingled sclerotic tissue, while the papillary carcinoma presenting a rather uniform structure. In 2 cases of papillary carcinoma, 1 case of adenocarcinoma and 1 case of carcinoma simplex, a squamous metaplasia was seen. A different histologic picture was met at the second operation (No. 22) or in the metastatic field (No. 21). In a case of carcinoma simplex (No. 23) a squamous metaplasia was seen in a metastasis of the cervical lymphnodes.

k) Grading of BVI (12 cases) and CSI (30 cases) on the table 1 was described according to following criteria: very few of doubtful on many slides (±), one by several slides (+), one to several on a single slides (++), rich findings on every slides (+++). There was a marked finding of BVI at the adenocarcinoma, fewer at the papillary carcinoma. CSI was discernible on both groups frequently. In lower degree (±), however, BVI and CSI were found at the precancerous adenoma. In 2 cases of metastasizing goiter, the primary tumor was not obtained.

l) Metastasis: In 6 cases of papillary carcinoma (30%) and 34 cases of adenocarcinoma (36%), there were metastases in the cervical lymphnodes. 5 cases with bone metastasis and 1 case with pulmonary metastasis were limited to the adenocarcinoma.

m) Postoperative fates: 1 case of carcinoma simplex is living 14 years after operation. In 1 case of adenocarcinoma a cranial metastasis occurred $10\frac{1}{2}$ years later. In both cases, BVI and CSI of the removed tumor were detected in a lesser degree. In spite of a marked CSI and lymphnodal metastasis, 7 case of papillary carcinoma (35%) and 2 cases of adenocarcinoma (18%) survived over 5 years. Accordingly, the postoperative fates of thyroid cancer should be regarded as tolerably good.

Comment

Histologic classification of the thyroid cancers was initiated by Langhans,[6] Wegelin[7] etc., while the so-called "benign metastasizing goiter" aroused much interest from workers. There is another problem difficult to dissolve on the papillary adenoma and the papillary carcinoma. Even a famous pathologist like Willis[8] stressed that both groups cannot be distinguished from either. Modern English writers, however, are inclined to recognize the importance of BVI and CSI discovered by Graham (1924,[1] 1925[2]), ascribing the cancerous nature to those.

According to Herbut,[9] in about one-half of the cases, a diagnosis of carcinoma is not even suspected until microscopic sections are studied. In such instances the microscopic appearance is said to be usually that of an ordinary adenoma. By examination of BVI and CSI in 185 cases of nodular goiters, the writer was also impressed with the fact, i.e. among

40 cases of thyroid cancers, 16 cases were not suspected of their malignancy from clinical observations.

Wilson[10] classified the thyroid cancers in 23 types, although such an idea would be regarded as nothing else but a criterion of the pleomorphism of it. A simple method as it was designed by Warren,[11] Crile,[12][13] Pemberton,[14] Dunhill[15] etc. would, therefore, be desirable for the sake of clinical convenience. According to Warren[11] and Crile,[12] the writer distinguished papillary carcinoma from adenocarcinoma, the latter including (a) and (b) type of adenocarcinoma described by Willis.[8]

Recurrence or metastasis of a thyroid adenoma of histologic benign nature was noted not infrequently by many workers (Ehrhardt,[16] Graham,[1][2] Warren,[17] and Dunhill[15]) emphasizing that a positive finding of BVI and CSI suggests a malignant character of the tumor, especially when it reveals a papillary structure (Willis[8]). In our case, 1 of papillary cystadenoma and 1 of parenchymatous adenoma died of a recurrent struma several years after operation, suggesting a malignant change in the ultimate end. For such a case of potential malignancy, the term, a precancerous thyroid adenoma, was selected. Whether a precancerous change may ever turn to a true carcinoma or not, it is not always advantageous to mind only the shape of a tumor cell itself for a diagnosis of cancer. At the present stage of histology, there is no decisive finding for a cancer cell. On the other hand, a destructive working against the milieu should be regarded as the greedy character of cancer cells. However, resembling a normal thyroid tissue, the destruction of a bone or metastsis of a tumor in the lymph node would be nothing else but the feature of cancers. In this respect, the nomination by Willis,[8] " a colloid containing vesicular adenocarcinoma " instead of a benign metastasizing goiter, would be accepted reasonably.

Blood affinity of cancer cells is the character simulating to a certain stage of embryonic cells, while glandular cells of the endocrine system have an especially intimate relation with the blood. Silver impregnation of goitrous tissue served the purpose of discretion of BVI, by presentation of an argyrophilic membrane of the capillary wall. In accordance with the invasion of the vascular wall by tumor cells, however, the limiting membrane under the endothelium changed the argyrophilic manner (degeneration ?), elastic fibers also disappearing prior to it. By falling of the red corpuscles from the routine paraffin section, a discretion of the dilated blood vessel in which the tumor cells are floating was not always easy, and to point out the lymphvessel was a more difficult work. Under consideration of communicating possibilities between tissue clefts and lymphatic spaces, however, the lymph vessel invasion by tumor cells should be included in the conception of CSI. In spite of marked CSI, there are

TABLE
40 Cases of Malignant

Case No.	Age	Sex	Previous goiter	Clinical symptom	Mode of operation	Naked-eye appearance				
						Affected lobe	Multiplicity	Size	Adhesion	Cyst
1	30	F	−	−	ENC	I	S	Hen egg	++	−
2	33	F	+	+	LET	L	S	Dove egg	+	−
3	35	F	−	+	LET	R	S	Over hen egg	−	+
4	35	F	+	+	LET	R	S	Over goose egg	++	+
5	36	F	−	−	PTE	L	S	Adult fist	++	+
6	37	F	+	+	LET	L	S	Over goose egg	+++	+
7	39	F	−	−	ENC	L	S	Dove egg	+	+
8	40	F	+	+	ENC	R	M	Small finger tip	+	−
9	40	F	−	+	ENC	L	S	Peas	−	−
10	40	F	−	+	EEC	L	S	Over walnut	+++	−
11	44	F	−	+	LET	R	S	Thumb tip	+	+
12	50	M	+	+	ENC	L	S	Dove egg	+++	+
13	50	F	+	+	LET	R	M	Hen egg	++	+
14	52	F	+	+	LET	L	S	Hen egg	+	−
15	52	F	+	+	PTE	R	S	Over dove egg	+++	+
16	52	M	+	+	ENC	LA	S	Over dove egg	+++	−
17	55	F	+	−	PTE	L	M	Dove egg	+	−
18	56	M	+	−	ENC	L	S	Adult fist	+++	+
19	60	F	+	+	PTE	L	S	Dove egg	+	−
20	64	F	−	+	PTE	L	S	Hen egg	+++	+
21	30	F	+	+	STE	LR	M	Goose egg hen egg	−	−
22	31	F	+	+	STE	R	S	Over hen egg	−	+
23	36	F	−	+	ENC	R	S	Walnut	−	−
24	42	F	−	−	ENC	L	S	Adult fist	−	+
25	47	M	+	−		L	?	?	?	?
26	50	F	+	−	PTE	L	S	Small apple	−	+
27	54	F	+	+	ENC	R	S	Over hen egg	+++	−
28	54	F	−	+	PTE	LR	S	Hen egg	+++	−
29	56	M	−	+	ENC	L	M	Adult fist	++	+
30	60	F	−	−	Head op.			No struma		
31	60	M	−	−	ENC	R	S	Over adult fist	−	−
32	36	F	+	+	PTE	LR	M	Walnut hen egg	+++	+
33	40	F	−	−	LET	R	S	Child fist	−	+
34	34	F	+	+	LET	L	S	Child head	+++	+

I

Goiters

Histologic F.				Postoperative fates	Histologic diagnosis
Structural variation	B.V.I.	C.S.I.	Metastasis		
−	±	##	−	2½Y living	Papillary carcinoma
+	##	##	−	1 Y living	
+	−	+	CLN	5Y lived; later unknown	
−	−	##	−	12M healthy	
+	−	##	CLN	8Y living	
−	−	##	−	5Y later recurred	
+	−	+	−	5½Y later recurred	
−	±	##	CLN		
−	−	##	−	½Y living	
−	##	##	−		
−	±	##	−	5Y living	
−	−	##	CLN	3Y later recurred	
−	−	##	−	8Y later recurred	
+	−	+	CLN	5Y living	
+	±	##	−	½Y later recurred	
−	+	##	CLN	½Y living	
−	±	##	−	1Y 10M lived	
−	±	##	−	½Y living	
+	+	##	−	7M later died	
−	##	##	−		
+	##	+	CLN Lung		Adenocarcinoma
+	−	+	−	10Y later cranial mt.	
+	##	##	Cranium	3M later headache	
+	−	+	−	2Y living	
+	+	+	CLN Sternum	2½Y later died	
+	?	?	Pelvis Femur	26D later died	
+	##	##	CLN		
+	±	##	−	unknown	
+	+	##	−	6Y living	
+	?	?	Cranium	Several hours after op. died	
+	##	##	CLN		
+	##	##	CLN	½Y later lung met.	C.S.
+	−	±	−	14Y living	
−	−	−	−	unknown	

35	41	F	+	+	Inop.	LR	?	Goose egg	##	?
36	43	F	+	+	ENC	R	S	Dove egg	+	+
37	60	M	−	−	LET	R	S	Over adult fist	−	+
38	69	F	−	+	LET	L	S	Hen egg	##	+
39	23	F	+	+	STE	LR	M	Peas	−	−
40	33	F	+	+	ENC	R	S	Hen egg	−	−

cases surviving several years after operation, of which the effect of removal of the primary tumor should be reminded. Pool's[18] idea is much too pessimistic on this problem.

Dominant frequency of paillary carcinoma over non-papillary adenocarcinoma in our cases is harmonizing with many writers' reports of this country (Inutsuka,[19] Iwaki,[20] Takada,[21] Nakamura[22] etc.) except one or two authors (Tosu-Nakajima[23]), although it sounds inversely to that of De Quervain,[24] J.M. Graham, A. Graham,[1][2] Pemberton[14] etc. As for the discrepancy between those different frequencies, De Quervain's opinion that in the goitrous district non-papillary malignant adenomas are met far more frequently than paillary adenoma, is interesting, as there are few districts of an endemic goiter in our country.

It is well-known that, owing to their pleomorphisms, no microscopic classification of the thyroid cancers is satisfactory. According to the writer's observation, however, the papillary carcinoma is rather monotonic comparing with frequent structural variations of the adenocarcinoma and carcinoma simplex. Pleomorphic appearance in the degree discernible in toxic goiters should be regarded as a characteristic of the thyroid gland. Squamous metaplasia was occasionally seen, while a hypernephroid mimicry (Boyd)[25] or a sarcomatous transformation was not observed. Remains of hyalinized sclerotic tissue and traces of calcification betray a malignant change on the previous nodular goiter, hemorrhagic necrosis and blood cyst reminding of an effect of BVI. Thus, structure of a malignant goiter may undergo secondary transformation, besides metaplastic changes of the metastasis (metastatic transformation) and structural variations at the second operation (post-operative transformation). At any rate, it would be rather difficult to determine the malignancy of thyroid cancers solely from the microscopic picture of a tumor cell.

The biological features of papillary carcinoma and adenocarcinoma are antipodal in various respects. Comparison of a distant result revealed a favorable condition of the former relatively (Table II). Concerning the age incidence, there is also a marked difference, as is shown in the Table III. Various factors are contrasted on Table IV. Accordingly, the writer intends to suppose a different origin of both types of thyroid cancers. Crile[12] stressed a congenital origin of papillary

+	−	±	−	5Y later died	Papillary cystadenoma
+	−	−	−	10Y healthy	
−	−	−	−	3Y later died	
−	−	−	−	6Y living	
+	±	−	−	6Y later died	P.A.
−	?	−	−	2Y living	

carcinoma. In our cases, one of papillary carcinoma suggested an origin from the left aberrant lobe apparently.

On Table I, clinical symptoms were not noted precisely. Comparing the symptoms of cancer cases with that of 145 benign nodular goiters (Table V), dyspnea and dysphagia are dominant on the cancer, although not conclusive. Thus, clinical symptoms alone would not serve the purpose of cancer diagnosis.

TABLE II

Distant Results of 2 Types of Thyroid Cancers

Classification	Obtained answers	6M			1Y			3Y			5Y			10Y		
		Death	Recurrence	Survival	Death	Recurrence	Survival	Death	Recurrence	Survival	Death	Recurrence	Survival	Death	Recurrence	Survival
Papillary Carcinoma	15	0	2	13/15 87%	1	1	9/11 82%	1	0	6/7 86%	1	1	5/7 71%	0	1	0/1
Adeno-carcinoma	7	2	0	5/7 71%	2	0	4/6 67%	2	0	2/4 50%	2	0	2/4 50%	1	1	0/2

TABLE III

Age Incidence of Various Types of Thyroid Cancers

Types \ Decades	0–10	11–20	21–30	31–40	41–50	51–60	61–70
Papillary carcinoma	0	0	1	8	3	7	1
Adenocarcinoma	0	0	1	2	3	5	0
Carcinoma simplex	0	0	0	2	0	0	0
Precancerous adenoma	0	0	1	2	2	1	1
Total	0	0	3	14	8	13	2

The size of a nodule does not always depend upon its malignancy. Occasionally, a very small nodule not palpated clinically makes a remote metastasis. On a case of adenocarcinoma metastasized in the cranium, there was no struma clinically, and a primary focus in the thyroid was

TABLE IV

Comparison of 2 Types of Thyroid Cancers

Factor \ Type	Papillary carcinoma	Adenocarcinoma
Consistency	Hard	Firm
Surface	Knotty	Smooth or not smooth
Adhesion	Marked	Not so marked
Cyst	Occasionally large	Small, if ever seen
Capsule	Thick	Thin
Interstitium	Well developed; dendritic	Scanty, except central cicatrix
Pleomorphism	Rather uniform	Marked
Blood vessel	Thick-walled; scanty	Thin-walled; abundant
BVI	Rather rare	Frequent
CSI	Very marked	Not so marked
Age of frequent affection	30 decade	50 decade
Metastasis	Lymph node	Bone, lung and lymph node
Prognosis	Good	Worse
Supposed origin	Papillary adenoma	Parenchymatous adenoma

TABLE V

Comparison of Symptoms of Benign Adenoma Thyroid Carcinoma

Classification \ Symptom	Pressure feeling of the neck %	Dysphonia %	Dysphagia %	Dyspnea %	Loss of weight %	Toxic symptom %	Pains %
Benign nodular goiter (145)	29	9	9.7	8.3	16.2	29	7.6
Carcinoma (33)	33.3	24.2	24.2	3	12.1	21.2	12.1

not even suspected until microscopic examination was performed. Another case of papillary carcinoma diagnosed as a benign adenoma revealed under microscope a small nodule (2×2 mm), presenting a marked CSI and infiltrative growth into a normal thyroid tissue already.

CONCLUSIONS

1. Clinical symptoms alone do not serve the purpose of diagnosis of malignant goiters. On histologic examination of 185 cases of nodular goiters, 40 cases of thyroid cancers were detected, of which 16 cases were not even suspected of their malignancy from clinical observations.

2. Blood vessel and capsule invasions (Graham) should be regarded as a diagnostic criterion on thyroid cancers.

3. 40 cases of thyroid cancers were classified as follows: Papillary carcinoma 20; Adenocarcinoma 11; Carcinoma simplex 2; Precancerous thyroid adenoma 7 (papillary cystadenoma 5 and parenchymatous adenoma 2).

4. Biological differences between papillary carcinoma and adenocarcinoma were discussed.

I wish to express my sincerest thanks to Prof. Nasu and Dr. Kurobane of the Pathological Department for their advices on the histological investigations.

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Nucleic Acids

I. Nucleic Acids of Hog Spleen

First Paper

By

Kiyosi Ogawa
(小 川 清)

(From the Medico-chemical Institute, Tohoku University, Sendai; Director: Prof. H. Masamune)

(Received for publication, December 22, 1951)

It is generally not easy to prepare PNA and DNA from an organ without any contamination of each other. And even of recent investigators of spleen DNA, Petermann & Lamb[1] and Chargaff *et al*,[2] the former group could not separate it entirely pure in this respect although a fine-devised method was applied. As to the spleen PNA none appears to have isolated it.

In the present work, isolation of DNA in the highly associated state was not intended so that slight alkalization or acidification of its solutions was dared to simplify the procedure. Hence only low-viscous fractions of DNA were yielded. All PNA was carried down at pH 9.4 together with a part of DNA by baryta and from the deposit, dried for several days, could the former be separated from the latter by repeated extractions with 10% NaCl. The preparations purified were either DNA or PNA and not mixtures of those two kinds of nucleid acid, but one of the fractions of DNA, whose amount allowed electrophoretic examination, proved inhomogeneous. The crop of PNA was less than 10% of that of DNA in any run.

To particularly mention, this account introduces a new expedient method of preparing pure pentose nucleic acid. Despite of their admirable means Kerr and Seraidarian[3] was not successful in obtaining pancreas-PNA free from DNA. Besides it is worthy to note that neither glucidmin nor carbohydrate had come into saline (10%) extracts of the tissue.

Experiemental

Preparation Procedure

A typical example will be given.

The preparation was carried out during winter in a cold room (<5°

C). Hog spleens quickly brought from a slaughter house were freed at first from adipose tissue and then from blood by perfusion of physiological saline through a. lienalis. 14 fresh spleens thus treated and weighing 1,450 g. were masticated and shaken with three volumes of 10% NaCl for two days. Percolated and filtered. The filtrate—**Fluid I**—was an extremely viscid, red-tinted and transparent fluid of pH 6.8. The tissue residue was further subjected twice to a similar extraction. The final residue (87 g.) contained a trace of phosphorus but no purine ($AgNO_3$-NH_3 test).

The successive extracts were acidified each, when filtered, by addition of 10% HCl carefully to a pH of 1.4, whereby maximum precipitation occurred. The following description concerns the first extract. The precipitate (acid-insoluble part) was separated from the mother fluid—**Fluid II**—by quick centrifugation and shaken with an equal volume of 10% NaCl, adjusting the pH of the mixture to 7.0 with 0.1 *N* NaOH, for 6 hours. The centrifugate was manipulated twice more in a like manner, extending over 12 and 24 hours respectively, when no more purine remained in the residue. The solutions were dialyzed together against running water for two days and distilled at low temperature to about 20 cc. and the saturated $Ba(OH)_2$ was added in drops to pH 9.4. The abundant deposit centrifuged (The mother fluid will be referred to as **Fluid III**) was removed from the excess baryta by stirring up with portions of a dilute HCl of pH 1.8, until a nearly neutralized washing was given, further washed with alcohol and ether and dried in a vacuum-desiccator over $CaCl_2$ for 5 days. 1,430 mg. of a white powder were given. The corresponding products from the second and third extracts amounted only to 24 and 5 mg. respectively. All of the products at this stage were biuret-negative, but Dische (for DNA)-, Bial (for PNA)- and Molisch-positive.

They were combined and kneaded with 10% NaCl (0.5 cc. per 100 mg.), adding 0.1 *N* HCl to maintain the pH at 7.0, and centrifuged. On the undissolved was repeated the extraction again and again as long as the residue showed the positive Molisch reaction (The supernatant combined—**Fluid IV**—were set aside for obtaining PNA. See below). Next, the operation was resumed, replacing 10% NaCl with 3 *M* NaCl. Almost 20 repetitions were here requisite to reduce purine to practically nil. The second solutions (in 3 *M* NaCl) were dialyzed for three days against running water and condensed (by distillation in vacuo to about 15 cc.) and 3 volumes of alcohol added. And the precipitate was reprecipitated from water with alcohol twice. To change the Ba,Na-salt into free acid, it was washed with a mixture of 99 volumes of methanol and 1 volume of 16% HCl three times. It was further washed with alcohol and ether

and after drying, the white powder obtained was taken up in a smallest possible quantity of water by the aid of NaOH (The pH was not raised above 8.0), followed by precipitation with two volumes of alcohol and thorough washing with alcohol. Finally a trace of the insoluble part was eliminated by redissolution of the substance in water (5 cc.), centrifugalization and precipitation of the supernatant with 1.5 volumes of alcohol[4]. The deposit was washed with methanol and ether. The Na-salt given—**DNA I** (a white powder)—amounted to 225 mg. It formed a low-viscous neutral solution in water and was also soluble in 97% glacial acetic acid. Molisch negative, Bial faintly positive, Dische distinctly positive. Purine and pyrimidine were detected (Ag-test).

Fluid IV that contained a minute quantity of DNA besides PNA was dialyzed for two days, distilled in vacuo to about 10 cc. and precipitated with 3 volumes of alcohol. The precipitate (crude Ba,Na-salt of PNA) was treated, with the aim of obtaining free acid, like as the corresponding salt of DNA above, then reprecipitated twice from a little water (A very small insoluble part that consisted of DNA was discarded) with methanol and washed with methanol and ether. 18 mg. of a white powder were yielded—**PNA**. It gave a slightly acid and slightly viscous solution in water, but did not dissolve in 97% glacial acetic acid. In 1 *N* HCl it dissolved into a milky solution (Centrifugation separated no sediment). Bial and Molisch distinctly positive but Dische negative. Presence of purine and pyrimidine was demonstrated.

On acidification of Fluid III (the supernatant fluid of the baryta-precipitate of DNA and PNA when the first tissue extract was fractioned) with HCl, there happened a voluminous precipitate at pH 1.4, which was centrifuged, washed with alcohol and ether. 865 mg. was the product. Those from the second and third extracts weighed 66 and 20 mg. They were strongly biuret-positive and faintly Molisch-positive. A trace of phosphorus was detected but no purine. Osaki-Turumi tests (the direct as well as the indirect) were negative and an acid hydrolysate scarcely gave Elson-Morgan reaction. Neither Neuberg-Saneyoshi nor Goldschmiedt was shown. All of the three acid mother fluids were dialyzed, evaporated and precipitated with alcohol. The deposits were washed and dried as above. Yields, 465, 45 and 36 mg. respectively. These were entirely devoid not only of purine but also of phosphorus. Biuret remarkably positive but Molisch negative.

Fluid II (the mother fluid of the acid precipitate from the first tissue extract) was evaporated at low temperature to about 15 cc. and as much 20% $CuCl_2$ was added as to produce maximum precipitation (The pH was lowered to 4.2). The centrifugate (The supernatant will be referred to as **Fluid V**) was washed with alcohol and ether. 390 mg. was the substance given (Cu-salts), which was biuret-positive and faintly Molisch-positive. From the mother fluids after acidification of the second and third tissue extracts were given 45 and 9 mg. respectively.

The products were united, well washed with a dilute HCl of pH

1.4 to perfectly liberate from Cu (The precipitate from the washings by alcohol was biuret-positive and Molisch-negative but contained no purine) and then agitated with portions of 3 *M* NaCl. The extracts here were dialyzed together, condensed and after precipitation at pH 9.4 with baryta as above, quickly centrifuged. The centrifugate that was biuret-negative was exhausted, employing 2~3 cc. portions of 3 *M* NaCl and when no more purine was detected in the residual, the solutions were combined and worked as when the sodium salt of DNA above (DNA I) was prepared from the corresponding solutions. The yield of the product—**DNA II**—was 13 mg. This showed resembling solubilities and chemical qualitative properties as DNA I. The centrifuged supernatant fluid of the baryta-precipitate was dialyzed and 5 volumes of alcohol were added. Some precipitate occurring showed the positive biuret reaction but negative Molisch and $AgNO_3$-NH_3 (for purine) test.

Fluid V (the mother fluid of the $CuCl_2$-precipitate in the run started with the first tissue extract) was dialyzed and after distilling in vacuo to about 10 cc., precipitated with 3 volumes of alcohol, and freed from Cu with changes of alcohol containing a small quantity of 10% HCl. The unidssolved was washed with alcohol and ether and dried. 34 mg. was the substance obtained. In the second and third runs 19 and 8 mg. were the crops. Purine and phosphorus absent; Molisch faintly positive; biuret distinctly positive. Osaki-Turumi reactions: the direct negative, the indirect faintly positive. Neuberg-Saneyoshi and Goldschmiedt, negative. An acid hydrolysate gave only faint Elson-Morgan reaction.

TABLE I

Composition of Nucleic Acids Separated from Hog Spleen

	DNA I* (sodium salt)	DNA II* (sodium salt)	PNA
N† (%)	10.3	10.4	11.0
P‡ (%)	5.9	5.8	6.4
N/P	1.7	1.8	1.7
Ash (%)	21.7§	20.0§	4.2//

* Ba was not detected. † Micro Kjeldahl. ‡ A modification of Plimmer method.[5] § Pregl's micro method. // Pregl's micro method without the use of H_2SO_4.

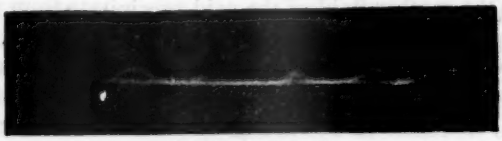

Fig. 1. An electrophoretic pattern of a 1% solution of DNA I (See the text) in phosphate buffer, pH 7.8, I=0.29; 20°C; mobility of the faster moving boundary 17.0 $cm^2 \cdot sec^{-1} \cdot volt^{-1} \cdot 10^5$. Descending limb to the right.

Composition and Homogeneity of the Nucleic Acids

Composition. See Table I.

Electrophoresis of a solution at pH 7.8 of DNA I demonstrated two components (Fig. 1).

Through the Grant Committee of the Science Council was given a grant from the Education Department, which enabled us to carry out this work.

H. M.

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Studies on the Defense Mechanism of the Body and the Neurohumoral Regulation

Report III. On the Relation between the Hormonal Secretion of the Adrenal Cortex and N. Splanchnicus

By

Shigeo Okinaka, Kiku Nakao, Mitsuo Nishikawa,
(冲中 重雄) (中尾 喜久) (西川 光夫)
Masao Ikeda and **Hiroshi Ibayashi**
(池田 正男) (井林 博)

(From Prof. Okinaka's Clinic, University of Tokyo School of Medicine, Tokyo)

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In the previous paper we reported that the stimulation of the N. splanchnicus resulted in the acceleration of antibody mobilisation and in the marked qualitative and quantitative changes of the blood picture, especially lymphocytes, in the previously immunized animal, and that these findings became far less evident in the animal whose adrenal veins had been ligated.

Through these experiments our attention has been focused to the role of the adrenal gland and N. splanchnicus in the immunization phenomenon. It has already been acknowledged that the hormonal function of the adrenal cortex is controlled by the anterior pituitary gland. Therefore, the adrenal cortex is said to receive purely the hormonal control. The neural control of the adrenal medulla is, on the other hand, a pattern of the mutual close relation between nerve and hormonal gland. For instance, the stimulation of the splanchnic nerve accelerates strongly the secretion of epinephrine substance from the adrenal medulla.

According to Long, however, the adrenal medulla has presumably a certain effect on the anterior pituitary gland through its epinephrine substance. Considering these facts, it is highly possible that the splanchnic nerve, adrenal cortex and medulla, and the anterior pituitary gland have functionally close connections with each other directly or indirectly.

Through the previous experiments we have come to entertain an idea that the splanchnic nerve might have some influence functionally on the adrenal cortex presumably in direct way.

So we tried first to know whether or not the chemocorticoid level (adreno-cortical hormone) in the adrenal veins is effected by the stimulation

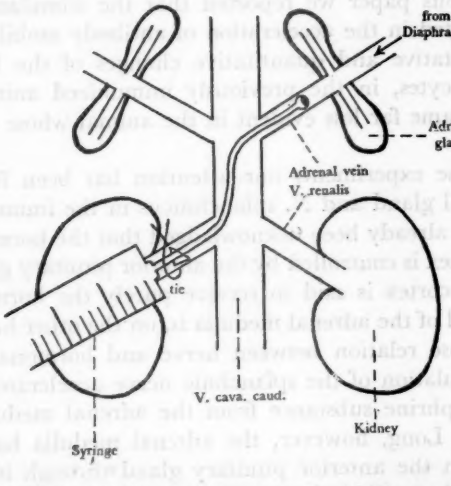
of N. splanchnicus.

Dogs weighed 6.7–11.5 kg. were used under morphine-narcosis (2.0 % morphin. hydrochlor. 0.5 cc. per kg. subcutaneously).

We stimulated the N. splanchnicus on both sides (experiment 1), on the left side only (experiments 2–6) and on the right side only (experiments 7–9), respectively at its sectioned peripheral stump, with induction current (6 volts, coil distance 6 cm.). The stimulation was repeated 10 times (each consisted of fifty seconds stimulation and ten seconds pause).

Before and after the stimulation the blood was obtained from the adrenal veins and the chemocorticoid concentration was estimated by means of the modified Heard- Sobel's method. As we needed 5 cc. of adrenal venous blood every time we had to obtain it by means of our newly designed injection syringe. We inserted this injection-syringe with a special made needle from the right renal vein into the left adrenal vein through the inferior vena cava (Fig. 1), and thus we could obtain the needed amount of adrenal venous blood with comparative ease.

Fig. 1. Blood collectionstechnique from left adrenal vein in dog.



Procedure of Blood Chemocorticoid Determination

Reagents

1) Protein precipitants, 0.2 N. barium hydroxide, 5% zincum sulfuricum
2) 2% sulfuric acid
3) Petroleum ether, boiling point, 30 to 60° C redistilled
4) Chloroform, chemically pure, redistilled

5) 0.1 N. sodium hydroxide
6) Sodium sulfate, anhydrous
7) Ethanol, chemically pure, redistilled
8) 0.1% Sodium carbonate anhydrous
0.2% Potassium ferricyanide
0.1% Ferric chloride (3% glacial acetic acid solvent)

Extraction and Determination of Reducing Substance (Chemocorticoid) of Blood

1). Protein precipitants, each 20 cc. of 0.3 N. barium hydroxide, and 5% Zincum sulfuricum are employed and the protein precipitate is removed by filtration.

2) The filtrate is brought to about pH 1.0 with 2% sulfuric acid, about 10% volume of petroleum ether is added and the mixture is shaken for about two minutes with a view to remove the blood lipids. This manipulation is repeated five times by separatory funnel, thus lipidfree filtrate is obtained. The petroleum ether washings are then discarded.

3) The lipidfree filtrate is extracted five times with 80 cc. portions of chloroform by shaking for five minutes with each portion. The filtrate layer is discarded.

4) This extract is washed twice with 10 cc. of 0.1 N. sodium hydroxide and once with distilled water. Each washing is backextracted with a little chloroform. These extracts are added to the former washed chloroform extract, the sodium hydroxyde and distilled water washings are then discarded.

5) This chloroform extract is dehydrated by sodium sulphate anhydrous and filtered through the glass filter into the round bottomed distillation flasks of 150 cc. capacity.

6) The clear washed and dehydrated choloroform extract is evaporated in vacuo at less than 60°C. in an all-glass distillation apparatus to dryness. Then the dried residue is obtained.

7) This dried residue in flask is dissolved with 3 cc. of redistilled ethanol. When the residue is thoroughly wetted, 1 cc. of this solvent is put into a test tube, and then 1 cc. of 1% sodium carbonate is added, followed immediately by 1 cc. of 0.1% potassium ferricyanide. Reagent blank is prepared from ethanol, 1% sodium carbonate and 0.1% potassium ferricyanide reagents equal volumes to those used in analysis. Immediately the tubes are placed in boiling water bath exactly for ten minutes. Then rapidly cooled to 25°C, and 1 cc. of 0.1% ferric chloride (3% glacial acetic acid solvent) are respectively added.

8) The resultant blue color density represents corticosteroid-like substance in the extract of blood. Color density is measured at 660 mμ by electrophotometer. The amount present is found by reference to

a calibration curve prepared from oxidation and colorimetry of desoxycorticosterone acetate (DOCA) in redistilled ethanol.

The result is expressed as microgramms of DOCA per 10 cc. of adrenal vein blood.

By the way we estimated urine-chemocorticoid content in various diseases by the above-mentioned method.

TABLE I

Urine Chemocorticoid Content in Various Diseases

Name	Sex	Age	Disease	Chemocorticoid mg. per day
T.	Female	26	Cushing's syndrome (adeno-carcinoma of adrenal gland)	8.25
Y.	Female	25	Cushing's syndrome (adenoma of adrenal gland)	6.58
K.	Female	36	Cushing's syndrome	5.50
K.	Female	75	Healthy old person	3.42
N.	Male	35	Healthy	3.19
S.	Male	25	Pleurisy with effusion (stadium of recovery)	2.75
O.	Female	33	Erythematodes	1.42
T.	Female	18	Simonds' disease	0.83
T.	Female	18	"	0.14

DOCA 100γ+distilled water 100cc. (pH 7)→68γ
DOCA 100γ+distilled water 100cc. (pH 1)→85γ

As the Table I shows, we can notice a remarkable increase of urine-corticoid in a case of Cushing's syndrome with adrenal adenocarcinoma, and a marked reduction in some cases of Simmond's disease on the contrary. The percentage of collection of chemocorticoid from DOCA 100γ (Desoxycorticosterone acetate) is about 85%.

Result

In the experiment 1 (stimulation of N. splanchnicus on both sides) and experiment 2–6 (stimulation of N. splanchnicus on the left side) the chemocorticoid content in the adrenal venous blood on the left side began to increase, amounting to the maximum between 2.5–25 minutes after the end of stimulation, and then returned to the former level gradually, as the table 2 and fig. 2 indictate.

The reason of the fact that the former chemocorticoid value and the maximum value after the stimulation are somewhat different in each case is presumably to be attributed partly to the fact that the obtained blood from the adrenal vein is inevitably mixed more or less by the blood in the vena cava inferior.

TABLE II

Chemocorticoid Content in the Left Adrenal Vein in Dogs; Effect of N. Splanchnicus Stimulation

(γ per 10 cc. adrenal vein blood)

Stimulated side \ Time (min.)	0	2.5	5	10	15	20	25	30	35
Exp. 1 (both sides)	40			422		374			
Exp. 2 (left side)	62	112		44					
Exp. 3 (left side)	38			68			156	62	
Exp. 4 (left side)	166	220		700				232	100
Exp. 5 (left side)	132		159	135				96	
Exp. 6 (left side)	71		80	176		686		56	
Exp. 7 (right side)	60				22	24		42	
Exp. 8 (right side)	22			40	20	30		10	
Exp. 9 (right side)	24		48	40			30	30	

The chemocorticoid substance in the left adrenal venous blood (non-stimulated side) in the experiment 7–9 (stimulation of the right N. splanchnicus) showed no noticeable increase in every case (the dotted curve in the Fig. 2).

DISCUSSION

In the first series of experiment we stimulated the left (or left and right simultaneously) splanchnic nerve and estimated the chemocorticoid content in the adrenal venous blood on the stimulated side. And we confirmed a distinct increase of chemocorticoid substance in the venous blood from the adrenal gland on the stimulated side.

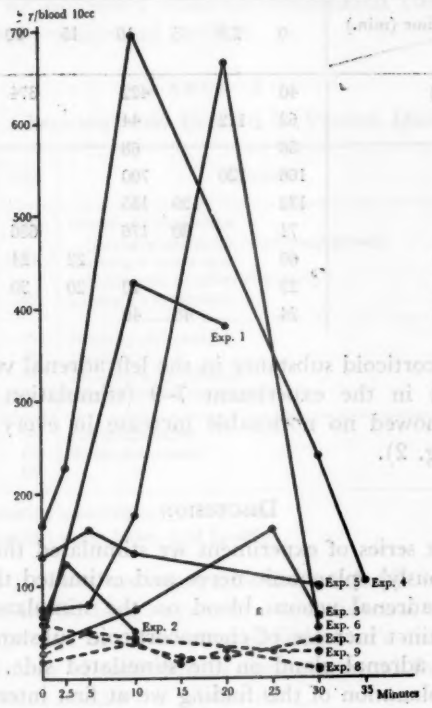
For the explanation of this finding we at first interpreted as follows: the increased epinephrine substance liberated from the adrenal medulla by the stimulation of the N. splanchnicus (this phenomenon can be easily understood) activates the anterior pituitary gland through blood circulation, releasing the adrenocorticotrophic hormon (ACTH) which again in turn stimulates hormonally the adrenal cortex. The increased chemocorticoid in the adrenal vein on the stimulated side can be thus hypothetically explained, because, the direct nervous control by the N. splanchnicus over the adrenal cortex has not been yet substantiated at present.

The second series of experiments (experiments 7–9) was carried out to solve our above said hypothesis.

If the stimulation of the N. splanchnicus causes an increase of chemocorticoid from the adrenal cortex indirectly through the epinephrine liberation from the adrenal medulla, acting on the anterior pituitary gland

Fig. 2. Chemocorticoid content in the left adrenal vein in dogs: Effect of N. splanchnicus stimulation (Exp. 1 both sides; Exp. 2–6 left side; Exp. 7–9 right side).

(γ per 10 c.c. adrenal vein blood.)



as we above suggested, the chemocorticoid in the adrenal vein on the non-stimulated side must be increased too, because the adrenal cortex is to be stimulated naturally on both sides hormonally from the anterior pituitary gland in this way.

The experiments 7–9 disclosed, however, that the chemocorticoid substance in the adrenal vein on the non-stimulated side showed no increase against our anticipation.

We are now continuing our studies on this problem, but, at present we must think over the existence of the direct nervous control of the adrenal cortex by N. splanchnicus from these results.

The mutual correlative functions of the pituitary and adrenal glands are recently considered as a topic of interest concerning the defense

mechanism of the body. Our experimental findings suggest that there exists not only a humoral regulation in the defence mechanism, but also a neurohumoral control mechanism. We believe that our experimental data offers an important contribution to the problem, concerning the neurohumoral control of the adrenal gland which plays a significant role in the defense mechanism of the body.

Summary

1. The stimulation of the N. splanchnicus causes a remarkable increase of the chemocorticoid substance in the adrenal venous blood on the stimulated side.

2. The stimulation of the N. splanchnicus causes no increase of chemocorticoid substance in the adrenal venous blood on the other side.

3. We discussed the mechanism of the neurohumoral regulation between N. splanchnicus, adrenal medulla and cortex, and anterior pituitary gland, concerning the defense mechanism of the body.

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Rotatory Crossgraphy (Rotatory Cross Section Radiography) of Ventricles and Subarachnoid Cisterns*

(Studies on Rotatography. 6th Report)

By

Shinji Takahashi and **Seiichi Anzai**
(高橋 信次) (安齋 精一)

(From the Departments of Radiology and of Psychiatry, Hirosaki University School of Medicine, Hirosaki)

Junnosuke Obara
(小原 準之輔)

(From the Department of Radiology, Tohoku University Faculty of Medicine, Sendai)

(Received for publication, January 7, 1951)

Technique

24 adult persons were introduced by us about 100–130 cc of air into the lumbar space after the customary technique of the encephalography, and were rotatory crossgraphed. The apparatus and the technique at the operation were as reported in the former issue of this Journal.[2)3)] The exposure was performed under 80 kv of the tube terminal, 45 ma in the tube current, 9 seconds of the exposing time, 97 cm of the focus-body rotationtable distance and 45 cm of the body table-film table distance. Four cross sections were roentgenographed. Each of them was parallel to the base cross section which contained angulus oculi temporalis sinistra et dextra and porus meatus acusticus externus sinistra et dextra. They were named from 1st to 4th one. The x-ray image of the cross sections which are considered as normal will be described below.

Result

The First Cross Section which contains a point 2.5 cm upwards of the upper margin of the orbit. (At the center of ventriculus lateralis) (Fig. 1. A, B and C)

At the center of cranium there are imaged ventriculus lateralis sinistra et dextra which lie in pair and symmetrical with regards to the

* Presented at the 2nd Meeting of the Hirosaki Roentgen Society held on March 4th 1951.

Fig. 1

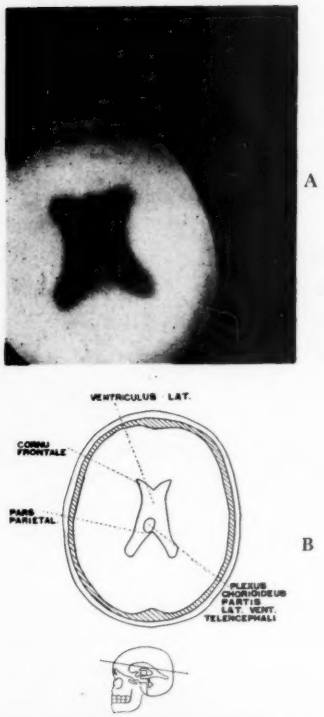

Fig. 2

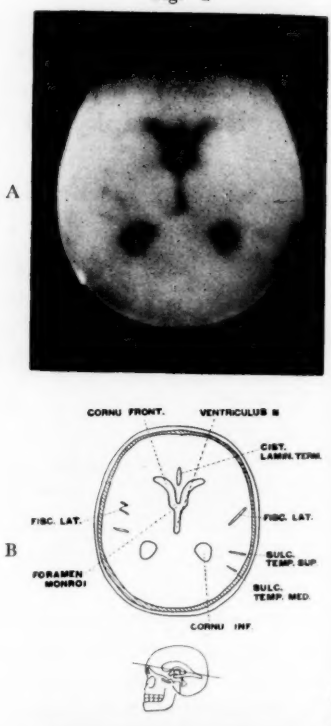

Fig. 1. A. Rotatory crossgram of the First Cross Section of the head.
B. Sketch of Fig. 1. A. Monography traced actually after the x-ray image. Crossgraphed position shown at the bottom.
C. Ventriculus lateralis in the actual cross section (arrow).

Fig. 2. A. Rotatory crossgram of the Second Cross Section of the head.
B. Sketch of Fig 2. A. Monography traced actually after the x-ray image. Crossgraphed position shown at the bottom.
C. Actual cross section. C: cisterna corporis cerebralis. One arrow: cornu frontale. Small arrow: third ventricle. Two arrows: root of pars temporalis ventriculi lateralis.

Fig. 3

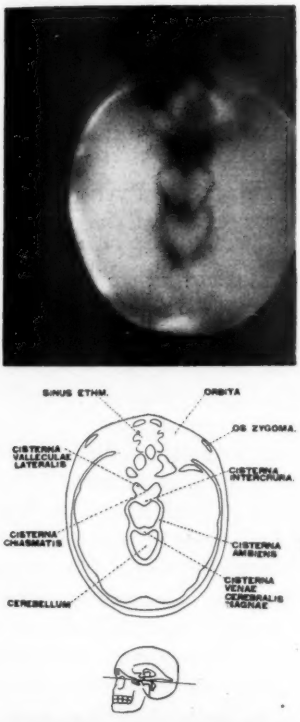

Fig. 4

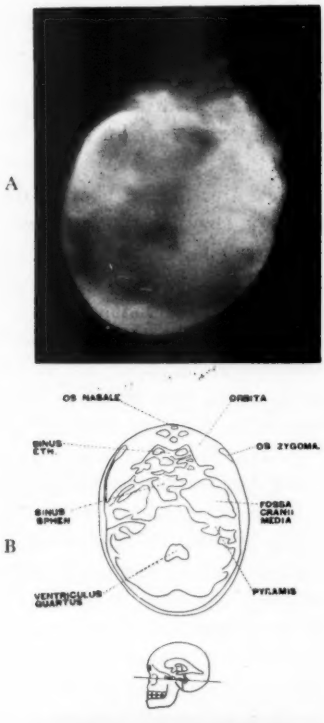

Fig. 3. A. Rotatory crossgram of the Third Cross Section of the head.
B. Sketch of Figur 3. A. Monography traced actually after the x-ray image. Crossgraphed position shown at the bottom.
C. Actual cross section. cerebellum (arrow).

Fig. 4. A. Rotatory crossgram of the Fourth Cross Section of the head.
B. Sketch of Figur 4. A. Monography traced actually after the x-ray image.
C. Cerebellum placed in the fossa occipitalis. Fourth ventricle (arrow).

median line. Its x-ray image looks like a letter of X drawn with a long trunk. The anterior parts, aparting each other, are cornu frontalia. The others are partes parietales. Septum pellucidum is imaged with difficulty.

The Second Cross Section which contains a point 1.5 cm upper than the upper margin of the orbit. (At foramen interventriculare (Monroi).) (Fig. 2. A, B and C)

At the inside of cranium there are imaged two kinds of ventriculus, namely, cornu frontale (front) and the root of pars temporalis ventriculi lateralis (back) and ventriculus tertius. The posterior part of cornu frontale and the anterior part of ventriculus tertius form together Y-shaped tubular connections, namely, foramen interventriculare (Monroi).

At the lateral of them there are imaged fissura cerebri lateralis, sulcus temporalis superior et medius. The small oval x-ray image, which is situated on the median line and is lain between cornu frontale, coresponds to cisterna corporis cerebralis under genu corporis callosi.

The Third Cross Section which contains the upper margin of the orbit. (At the subarachnoid cisterns of the brain base) (Fig. 3. A, B and C).

At the median there are arranged from front to back cisterna vallеculae lateralis cellebri, cisterna ambiens, cisterna venae cerebralis magnae and cavum leptomenigicum of monticulus cerebelli in this order. Borders of these cisternae, however, are not imaged. Cisterna chiasmatis forms V in figure with concavity to the front due to the oppression by chiasma opticus. Cisterna intercruralis, ambiens and venae cerebralis magnae connect together and form heart shaped x-ray image. The figur enclosed by these cisterns corresponds to mesencephalon. At the back of this figure Backwards this there is contoured the round cross section of the leptomenigen of cerebellum.

The Fourth Cross Section, namely, the Base one (At the fourth ventricle) (Fig. 4. A, B and C).

At the back in cranium there is an x-ray image of trapezoid 2.5×1.5 cm in size. This is the fourth ventricle. Due to the projection of nodulus cerebelli into the ventricle, the ventricle is a little concav to the back. At the Fourth Cross Section there is imaged sometimes cisterna pontis at the back of clivus. In the bony contour of cranium there are imaged os zygomaticus, sinus ethmoidei, the orbit and the pyramis.

The ventricles and cisterns are very complicated in shape. If we crossgraph them a little upwards or downawards from the numbered standard cross section, they are imaged with different appearances. In the cross section about 5 mm upper than of the First Cross Section, there are imaged small two partes parietales ventriculi laterales being separated 2 mm each other. If we incline the second cross section about

10 degrees downwards at the back, we obtain the cross section of the wide cornu frontale and the vigorous third ventricle with the center defect due to massa intermedia of thalamus. At the Third Cross Section the figure of cerebellum changes its size rapidly when the position of roentgenography is a little up-or downwards set up. Moreover, we miss sometimes the contour of cerebellum when air has not entered in tentorium. Roentgenographed a little upwards than that of the standard, cisterna cerebralis venae magna is imaged wide with the x-ray image of the projection of recessus suprapinealis at its front wall. Accordinghy, if we wish to take a radiogram of the same cross section as shown in the figures presented, it might be preferable to take an ordinary encephalogram beforehand and then adjust the photographing-position to that of the ventricles or cisterns imaged on the encephalogram. When we operated the crossgraphy correctly, the x-ray images of ventricles or cisterns are arranged quite symmetrical with regards to the median line.

Discussion

Since Danbys[1)] first operation, the encephalography and the ventriculography have been applied widely to the psychiatry or brain surgery. The customary encephalography, however, presents the superimposed x-ray image or only two dimensional one. Accordingly, the interpretation of the x-ray image is sometimes difficult. The rotatory crossgraphy gives a picture of another dimension, that is, the depth. Only after adding the crossgraphy into the customary encephalography, we can obtain the three dimensional knowledge of the ventricles and cisterns.

We are indebted to Prof. Kyozo Kudo, Chief of the Department of Anatomy of the Hirosaki University, for his kind help.

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Liver Excretory Function Test with Bromphenolblue

By

Tamotu Numasawa
(沼澤 保)

(From the Medical Clinic of Prof. T. Kurokawa,
Tohoku University, Sendai)

(Received for publication, January 9, 1952)

On the dye used for the liver excretory function test, the results of their experiments have been published by many researchers, and Kaito and others[1] of our clinic have found that bromphenolblue (BPB) is also available for this test.

With this dye as its center I carried out clinical observation from various points of view, especially as compared with azorubin S (A–S) and some other liver function tests. Double loading method, as we may call it, in which a dye is given two times to the object, also was employed in my experiments.

Experimental

As BPB is slightly soluble in water, 1.5 cc. of 0.1 *N* NaOH was added to every 100 mg. of the dye to make the solution. Upon the basis of our preliminary experiment[1] 4.0 cc. of a 0.5% solution was intravenously administered and the result was, after the A–S test method, judged by the total percentage of the excreted amount in the urine to the injected amount of the dye. I call this the single (loading) method, for convenience. On the contrary, in the double (loading) method, after 2.5 cc. of a 0.5% solution (in A–S test, 2.5 cc. of 1% solution) had been intravenously administered, the urine was collected every hour for two hours and after the collection at the second hour the same amount of the solution was again injected and the urine was collected in the same way, then the percentage of the excretion was calculated as to each of the specimens obtained.

In parallel with this, A–S test, santsol test, Takata's reaction, Meulengracht's bilirubin index, thymol and zinc turbidity test, colloidal-red reaction, urobilinogenuria, Millon's reaction, etc. were carried out. As the standard of disturbance of the first two tests Kaito's[2] was adopted,

Results

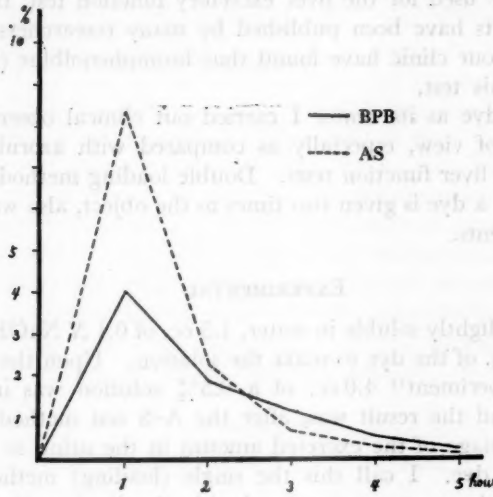
(I) Single method

A. *Control cases*

1) Observation on healthy persons. Results of 14 cases gave 5.56 to 10.06 as the total percentage of excretion, the average 8.62%. It required 4 to 5 hours for the dye to disappear from the urine. As to 6 cases, A–S test, which was conducted in parallel, showed the total percentage of 7.18 to 13.62, higher than that in case of BPB, the average reading 11.68%. The time required for the dye to disappear was somewhat reduced to 3 to 5 hours.

Fig. 1 shows the average value of the excreted amount of each of the

Fig. 1. Excretion of BPB in control cases (single method).



both according to the process of time. The value is highest at the first hour in both cases, then gradually lowering, but, as mentioned above, the excreted amount of BPB is small and its curve drops slowly.

2) Continuous observation on the same healthy persons. For two persons the BPB test was applied six times at intervals of 3 to 7 days and the result obtained was that in each case there was found very little fluctuation in the readings of excretion, the greatest difference being only 1.67% in one case and 2.01% in the other. Another person to whom A–S test was put also showed little fluctuation in the amount of excretion and the greatest difference was 2.54%.

From the foregoing it is known that in BPB test the normal value of the total excretion is in most cases below 10%, though there are some exceeding this percentage, and that more than 11% means pathological condition.

B. Cases with various diseases

1) Liver diseases. As to 16 cases including those with gall-bladder and -duct, BPB test and A–S test were carried out in parallel and the following results were attained (Table I): 14 cases were proved to be in

TABLE I

Results of Single Method in Cases with Liver Diseases

No. of case	Sex	Age	Diagnosis	BPB		A–S	
				Total output (%)	Disappearance time (hours)	Total output (%)	Disappearance time (hours)
1	M.	41	Hepatitis	70.92	24	51.05	24
2	F.	49	Hepatitis	58.12	24	68.88	24
3	F.	24	Hepatitis	65.63	24	53.38	24
4	M.	22	Hepatitis	18.96	7	30.93	5
5	F.	59	Acute phosphor intoxication	13.31	5	12.12	5
6	F.	20	Liver cirrhosis	10.28	5	8.94	3
7	M.	36	Liver cirrhosis	25.53	13	31.09	9
8	M.	20	Hepatoma	14.68	9	27.35	7
9	F.	43	Hepatic congestion	7.18	5	9.95	5
10	M.	47	Cancer of the bile-duct	69.40	24	52.88	24
11	M.	42	Cancer of the bile-duct	67.31	24	58.51	24
12	M.	57	Cholelithiasis	15.62	11	24.04	9
13	M.	40	Cholelithiasis	44.09	24	47.35	24
14	M.	51	Hydrops vesicæ fellæ	11.52	5	16.44	5
15	M.	45	Cholecystopathy	12.22	5	11.44	3
16	M.	21	Cholecystopathy	19.76	5	24.29	5

pathological condition through the BPB test (87.5%) and 12 cases through the A–S test (75.0%). Significant difference could not be recognized as to the percentage of disturbance, but the risk-rate of disturbance was 4.09% and interrelation was noticed between the both. It was observed too that in those cases of liver diseases there were some in which the amount of BPB excreted was rather larger than that of A–S, though in the control experiment it was to the contrary. As is shown in the table, in both tests

the grade of disturbance is in many of the cases paralleled with the amount of excretion. Many of the cases showing remarkable disturbance are accompanied by jaundice.

2) Other diseases. The results in 16 cases with digestive diseases, 12 with gastric and duodenal ulcer included, indicate that the tendency is, as is the same with healthy persons, that the excreted amount of BPB is generally small as compared with that of A–S and the dye is a little longer in disappearing from the urine. It was in one case that the BPB test showed a pathological amount, in which the A–S test also revealed disturbance. Through both tests 2 cases out of 4 with tuberculosis showed normal value, 4 cases with other diseases all giving normal readings.

In short, the above 24 cases gave an extremely good parallel relation as to the disturbance proved by the both tests.

(II) Double method

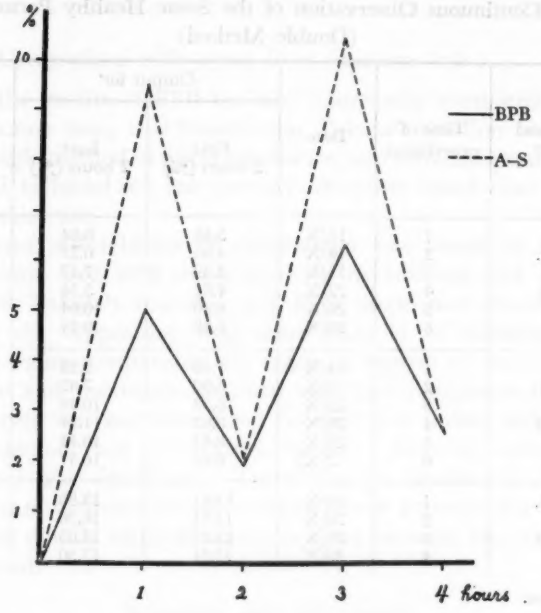
Kaito and others[3] advocated double method. They first obtained the percentage of the amount excreted in the first two hours (first percentage) and then the ratio to this percentage of that of excreted amount in the latter two hours (excretory ratio). With these two results they tried to judge the disturbance more sensitively. I further examined the significance of this method by employing BPB.

A. Control cases

1) Observation on healthy persons. The first percentages given by 16 cases extend from 4.70 to 8.77, the average 6.93, but in most cases they fall within the range of 6.0 to 8.0. The excretory ratios are 1.05 to 1.43, the average 1.29. Most of them lie between 1.15 to 1.35 and in only one case it is over 1.40. The results of 7 other cases to which A–S test had been applied were the same as given by Kaito and others.[3] In Fig. 2 were given the results of both tests in their average readings.

2) Continuous observation on the same healthy persons. As is seen from Table II, all cases give normal readings, except those of the 5th and the 6th time in Case 1 and of the 3rd time in Case 2, and the difference too is slight between every successive experiments. Case 1, who presented acute gastrointestinal symptoms owing to food intoxication on the day before the 5th experiment, exhibited a remarkable increase both in the first percentage and in the excretory ratio, the latter, though slightly, increasing towards the following 6th experiment. In the 3rd experiment in Case 2, it seems because he played baseball after he had received the second injection that the amount excreted conspicuously increased after the latter two hours and the excretory ratio suddenly rose. In 11 cases, next, the test by single method was applied in parallel with that by double method. Generally speaking, the injected dye, when small in amount,

Fig. 2. Excretion of BPB in control cases (double method).



tends to be excreted in large quantity in early hours and short in disappearing from the urine.

From the above results it is concluded that the normal value in the BPB test by double method is lower than 9.0% for the first two hours' reading and lower than 1.50% for the excretory ratio.

B. Cases with various diseases

1) Liver diseases. 28 patients, those with gall-bladder and -duct included, were put through this test. Among those 13 underwent the single method in parallel. The results were: disturbance was confirmed in 9 cases by double method and in 10 cases by single method. Both methods demonstrated pathological change for 7 cases. 1 case was given normal value by both tests, 3 by double method only and 2 by single method only. Therefore, as to the disturbance-rate there is no significant difference statistically; nor is there as to the interrelation of disturbance. Among 19 cases in which A–S double method was carried out in parallel, 14 showed disturbance through BPB test and 12 through A–S test. As to the disturbance-rate significant difference can not be recognized. It was in 11 cases that disturbance was told by both tests. 4 cases were

TABLE II

Continuous Observation of the Same Healthy Persons
(Double Method)

No. of case	Sex and Age	Time of experiment	Date	Output for		Excretory ratio
				First 2 hours (%)	Last 2 hours (%)	
1	M. 20	1	12/X	5.46	6.64	1.22
		2	14/X	4.95	6.27	1.27
		3	15/X	5.39	7.17	1.33
		4	22/X	4.61	5.54	1.20
		5	26/X	6.30	10.64	1.67
		6	29/X	6.38	9·24	1.45
2	M. 20	1	14/X	7.46	9.28	1.24
		2	15/X	5.90	7.35	1.25
		3	22/X	6.53	10.48	1.60
		4	26/X	8.67	11.34	1.31
		5	29/X	8.45	10.44	1.24
		6	2/XI	8.03	10.37	1.29
3*	M. 23	1	16/X	13.61	15.95	1.17
		2	20/X	13.74	16.76	1.22
		3	27/X	13.83	17.05	1.23
		4	30/X	13.91	17.30	1.24

* A–S test.

given normal readings by both, 1 by BPB test only and 3 by A–S test only. The risk-rate of the both was 3.67%, and so significant interrelation is thought to be.

2) Other diseases. BPB single method was employed in parallel with the double method of 23 patients—13 with tuberculosis, 4 with digestive diseases and 6 with other diseases. Double method showed disturbance in 10 cases (43.5%) and single method in 6 cases (26.1%). Statistically significant difference is not recognized as to their disturbance-rate nor interrelation of disturbance. Next, 28 patients, that is, 11 with tuberculosis, 13 with digestive diseases and 4 with other diseases, were put to this test in parallel with A–S double method. BPB test and A–S test proved respectively 10 (35.7%) and 6 (21.4%) to be pathological. The disturbance-rate is not significantly different.

Thus far, the results of tests were divided in two categories, liver diseases and other diseases. Now for total cases; the results of 47 cases tested by BPB double method and A–S double method gave the risk-rate 2.33%, which affirmed the existence of significant interrelation of disturbance. But, from the results of the tests made by BPB single method and A–S double method significant interrelation of disturbance is recogn-

ized as to the reading for the first two hours, but not as to the excretory ratio.

(III) Comparison with other liver function tests

When the results of BPB test are statistically compared with those of other function tests, it is known that Takata's reaction only is higher than BPB single method in disturbance-rate, and urobilinogenuria, Millon's reaction and colloidal red reaction are obviously lower than BPB test in relation to this rate.

Significant interrelation of disturbance was found to be between urobilinogenuria and BPB single method, thymol test and BPB double method, and Takata's reaction and BPB single and double methods. Further, on the supposition that more than 11 of bilirubin index be pathological value comparison was made in respect to the cases of liver diseases only and the result obtained was that significant difference in disturbance-rate was not recognized between this index and BPB single or double method, but interrelation between bilirubin index and BPB single method was significant. There was no significant difference in disturbance-rate or interrelation of disturbance between santsol test and BPB single or double method; nor was there between zinc test and BPB double method.

Summary and Discussion

As the results of their experiment with dogs Kaito and others[1] observed from various points of view that BPB could be used as a dye for liver function test.

This dye is fit for detecting the disturbance of liver function with voided urine, because in normal condition of health the greater part of it is eliminated from the liver and only a small portion from the kidney and in the liver disturbance an increased output in the urine is noticed.

Comparing with A–S which had been the only dye fit for urine examination, I made clinical observations from various points of view on this newly applicated dye, and found that in case of healthy persons the excreted amount of it was a little smaller than that of A–S and the time required for disappearance from the urine was prolonged. This is consistent with the experiment[1] carried out with dogs.

When continuous observations were carried out of the same healthy persons there was found rather less fluctuation in case of BPB than in case of A–S, in other words a strong stability, in the amount of excretion.

As to cases with liver diseases both tests, as mentioned above, showed parallel relation. In spite of normal value in A–S test, there are some showing slight disturbance in BPB test. And in some of the cases showing

remarkable disturbance excretion is more in BPB than in A–S, though in control cases the phenomenon is to the contrary. This suggests that BPB reacts sensitively when there is any distrubance.

Next, in case of other diseases the results of both tests are with remarkable significance parallel and the amount of excretion is, generally speaking, smaller in BPB, as in control cases.

Further, the meaning of double method has already been stated by Kaito and others,[3] and from the results of my experiment it may be said that those whose amount of excretion and excretory ratio are both pathologically abnormal are suffering from remarkable disturbance in the liver excretory function, and that abnormal excretory ratio means slight or latent disturbance of the liver, which rouses our deep interest when we consider together that food-intoxication or some exercise caused a remarkable rise in excretory ratio, as we see before. Especially that the disturbance proved by BPB and A–S double methods has significant interrelation indicates that both dyes can be used for the same purpose as in single method.

And from the results of some other liver function tests it is understood that BPB test has, as is mentioned above, acute sensitivity.

Conclusion

I made the liver excretory function test with bromphenolblue of healthy persons and of several kinds of patients by single and double methods.

1. In single method the normal reading of excretion is below 11% and the normal time for the dye to disappear is within 5 hours. In double method the normal reading for the first two hours is below 9.0% and the excretory ratio 1.50%.

2. When BPB and A–S tests are made of the same persons, the tendency is that if the person is in good health BPB is smaller in the amount of excretion than A–S and prolonged in the time required to disappear from the urine. But in case of liver or other diseases the disturbance is well paralleled with each other both in single and double methods.

3. BPB single method is suited for knowing the grade of damage of the liver function and double method is available for detecting latent disturbance of the liver function.

4. BPB is best suitable for the test of excretory functional capacity and others concerned, consequently is well worth clinically utilizing.

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On the Placental Substance Giving the Skin Reaction of Cancer and Pregnancy

First Paper*

By

Kiyota Oh-Uti, Masayosi Ogawa, Seiiti Yabuki
(大内 清太) (小川 政良) (矢吹 清一)
and **Hiroya Kawasaki**
(川崎 裕也)

(From the Medico-chemical Institute (Director: Prof. H. Masamune) and the Surgical Clinic of Prof. M. Muto, Tohoku University, Sendai)

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Matsubara[1] noticed that a placental extract, when applied intracutaneously, develops a more spacious redness in loco in cancerous patients and pregnant women than in non-cancerous patients and normal individuals exclusive of pregnant women. With the aim of establishing the chemical nature of the causative compound, the writers divided the watery extract of placentae, dehydrated with alcohol, by the aid of baryta and dilute and conc. acetic acid in succession, taking the outcomes of the skin reaction as an indication. In distinction from the other fractions, the glacial acetic acid-soluble showed definite difference of the width of the redness between the two groups of persons. Not only was this fraction free from nucleic acids but also from the free hexosamine-containing carbohydrates, which are generally insoluble in glacial acetic acid. Nevertheless, the electrophoretic pattern demonstrated several components. Since the fraction gave marked positive Molisch test besides the positive biuret, it was a mixture of ordinary simple proteins, involving glucidamins. To orient further experiments, its properties, chemical and plysical, were examined with results detailed in the experimental part.

Experimental

Fractionation Procedure of the Placental Extract

1) Fresh placentae that had been freed mechanically from amnion and decidua as far as possible were each cut into pieces, washed with water and placed in 10 volumes of denatured alcohol containing 3.5%

* Fourth Report of Masamune and coworkers' "On Proteins and Amino Acids."

methanol for 1 week or longer. Then they were squeezed, masticated and dried in a Faust apparatus. From one placenta were obtained 250 g. of the fresh chorion and 34.7 g. of the dried substance in averages.

A typical example will be given for the description of the following processes.

2) 500 g. of the dry material thus obtained were shaken with 5 *l.* of water (5 cc. of alcoholic 20% thymol solution were used as a preservative) for 2 days and strained through a cloth. The percolate was centrifuged. The tissue residue (the bulk on the cloth and the centrifugate) was subjected to a similar extraction twice more, employing 4.8 and 4.6 *l.* and continuing for 3 and 4 days respectively. The supernatant fluids obtained were 4.2, 4.6 and 4.5 *l.* and all neutral and strongly turbid. Each of them, soon on separation, was added to with the saturated baryta to a pH of 8.8~9.0, where maximum precipitation occurred (Sometimes barium acetate was added together). The centrifuged-supernatant was clear and had yellowish hue. The centrifugate was washed with two small portions of a dilute baryta of pH 9.0 and three 50~25 cc. portions of 99% denatured alcohol (methanol 3.5%) and dried in a desiccator. 2.9, 1.0 and 1.4 g. ($\mathbf{Ppt_{Ba(OH)_2}}$) were yielded from the successive extracts.

3) The mother fluids were combined with the respective dilute-baryta-washings and acidified with 30% acetic acid. The miximum precipitate occurring at pH 4.5 was washed with two 200 cc. portions of a dilute acetic acid of the same pH and dried after washing with alcohol as above—$\mathbf{Ppt_{ac}}$. Yields 1.5, 0.6 and 0.7 g.

4) In each run, the yellowish clear mother fluid and acetic acid-washings were combined and dialyzed against running water until no barium remained anymore. It took 3~4 days and the pH of the solution rose to 6.4~6.8. Some precipitate in the mean time was filtered off and the filtrate distilled in vacuo to a syrup, rejecting halfway some precipitate that had again appeared. And to the remainder was added about 50 cc. of the abs. denatured alcohol and the centrifuged deposit washed thoroughly with alcohol (Sodium acetate was used together, when necessary). 3.1, 2.7 and 2.05 g. were the yields. The products were a yellowish powder. Biuret and Molisch positive. Phosphorus detected.

5) These were united and taken up in 35 cc. of water (In most cases a slightly alkaline solution was given, which was neutralized with a dilute HCl) and the insoluble part was discarded after treating with several 10 cc. portions of water. The solutions (transparent, brown and slightly viscid) were distilled in vacuo and precipitated with alcohol and dried as above. Crop, 6.52 g.

6) To the substance given was added drop by drop of water to make

a homogeneous paste (About 2 cc. of water were required), and then 97% acetic acid added also in drops until a persistent precipitate appeared. Followingly 600 cc. of additional glacial acetic acid were added in larger portions when all the precipitable was carried down (In some examples, it was necessary to add anhydrous sodium acetate besides). The centrifugate was washed with a few 20 cc. portions of the glacial acetic acid with centrifugation. The supernatant liquids (the mother fluid and washings) were united, and precipitated with 4 volumes of ether (Additional ether gave no precipitate). The centrifugate was washed once with 50 cc. of abs. alcohol, next covered under 200 cc. of abs. alcohol overnight, and finally washed twice with 50 cc. of abs. alcohol, followed by drying (in vacuo over H_2SO_4). 3.25 g. of a yellowish powder were given—**Crude Active Fraction.** Biuret and Molisch positive, phosphorus detected.

The substance, precipitated and washed by the glacial acetic acid, was also freed from acetic acid by the aid of alcohol similar to above and dried. Yield 1.76 g.—**Crude Carbohydrate.** Biuret, Molisch and phosphorus (wet incineration followed by precipitation with ammonium molybdate) test all positive.

Preparatory Treatment of the Fractions for Skin Test and the Outcomes of this Test

Skin test. A weighed amount of the fractions operated as below was taken up in physiological saline (sterilized) and variously diluted with the saline and 1/9 volume of 5% phenol added so as to obtain a solution at 1:500~500,000. After the lapse of 4 hours from intracutaneous (forearm) injection of the solution (each 0.2 cc.), the longitudinal and lateral widths of the evolving redness were measured and their mean was calculated out to represent the area of the redness. In most cases the mean width of 25 mm. was taken for judgment of the test.

1) $Ppt_{Ba(OH)_2}$. a) 1 g. of it was suspended in 5 cc. of water and after addition of 10% HCl to a pH of 2.0, centrifuged. Since the supernantant was somewhat turbid, 10 mg. of crystalline barium chloride were added to the mixture again agitated. Centrifugation gave an almost clear supernatant fluid this time. The centrifugate here was washed twice with 5 cc. of a dilute HCl of pH 2.0. And to the mother fluid and washings united was added the saturated $Ba(OH)_2$ in small portions. The precipitates at pH's 4.5 and 8.0 were discarded. The pH was returned to 4.5 and the precipitate again occurring eliminated by centrifugalization. The supernatant was freed from barium with H_2SO_4, neutralized with NaOH and dialyzed. Finally the solution was distilled in vacuo to a syrup and precipitated with alcohol—90 mg. Biuret and Molisch positive. Phosphorus present.

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases Discrimination width 25 mm.	20 mm.
1:2,000	Cancerous	5	3	4
	Non-cancerous	11	4	5

b) To 800 mg. of the fraction ($Ppt_{Ba(OH)_2}$) were added 20 cc. of water and acidified with 10% HCl to pH 2.0. The insoluble part was washed with two 10 cc. portions of a very dilute HCl of the same pH and after thorough washing with abs. alcohol, dried. The powder given (750 mg.) was suspended in 5 cc. water, the saturated $Ba(OH)_2$ added to pH 9.0 and the centrifugate washed with a very dilute $Ba(OH)_2$ of pH 9.0 twice. The operations with HCl and $Ba(OH)_2$ were repeated twice and the baryta-precipitate obtained was washed with a very dilute HCl of pH 2.0, dissolved in water by the aid of NaOH (the pH was brought to 7.6) and after centrifuging off of the undissolved, dialyzed, followed by condensation and precipitation with alcohol. The product amounted to 100 mg. Biuret, Molisch and phosphorus test all positive.

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases Discr. width 25 mm.	20 mm.
1:2,000	Canc.	10	0	2
	Non-canc.	19	2	4

2) $Ppt_{ac.}$ 500 mg. of the substance were taken up in 10 cc. of water, 10% NaOH added cautiously to pH 9.0 and centrifuged. The clear supernatant was acidified with 30% acetic acid to pH 4.5. The precipitate was centrifuged, washed with two 10 cc. portions of a dilute acetic acid of that pH and three 40 cc. portions of abs. alcohol and dried. Yield 120 mg. Biuret and Molisch positive. Phosphorus present.

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases (discr. width, 25 mm.)
1:500	Canc.	11	10
	Non-canc.	10	9
1:1,000	Canc.	12	7
	Non-canc.	10	3
1:4,000	Canc.	10	3
	Non-canc.	11	3
1:10,000	Canc.	9	1
	Non-canc.	10	0

3) Crude Carbohydrate. This was applied to the skin test im-

mediately.

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases Disc. width 25 mm.	Number of positive cases Disc. width 20 mm.
1:2,000	Canc.	20	15	19
	Non-canc.	19	8	15

4) Crude Active Fraction. The whole of the product in 6) of the former section was taken up in 40 cc. of water and the saturated $Ba(OH)_2$ added in drops to pH 7.0. The insoluble part centrifuged. To the supernatant (yellowish-brownish and transparent) was added additional baryta to pH 9.0 and after centrifuging off of the precipitate, the supernatant was brought back to neutral and further changed to pH's 6.0, 4.5, 3.8 and 2.0 in turn with HCl, rejecting the occurring precipitate at each step. The solution was subjected twice more to the baryta- and HCl-treatment (In the second repetition no precipitates were given) and the final solution at pH 2.0 was liberated from barium with just enough sulfuric acid. After adjusting to pH 4.5 with 10% NaOH, it was dialyzed (0.5 cc. of toluene added for avoiding putrefaction) against running water as long as barium remained (Sometimes a little precipitate appeared, which was removed) and distilled in vacuo. The thick solution was precipitated with a mixture of equal volumes of abs. alcohol and ether and dried—**Purified Active Fraction** (1.5 g., a yellowish fine powder). Biuret and Molisch positive. Either of the direct and indirect Osaki-Turumi reactions resulted in slightly positive. Phosphorus was detected.

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases (disc. width, 25 mm.)
1:1,000	Canc.	12	11
	Non-canc.	14	7
1:2,000	Canc.	80	75
	Non-canc.	117	19
1:5,000	Canc.	10	7
	Non-canc.	8	1

Properties of the Purified Active Fraction

Quantitative analysis

N (micro Kjeldahl) 11.8%; hexosamine (Hamasato-Akakura) 4.7%; non-amino sugars as mannose: orcinol method 6.5%, indole method 7.7%, diphenylamine method I some 5.9%[2]; ash (micro Pregl) 9.1%.

Optical rotation

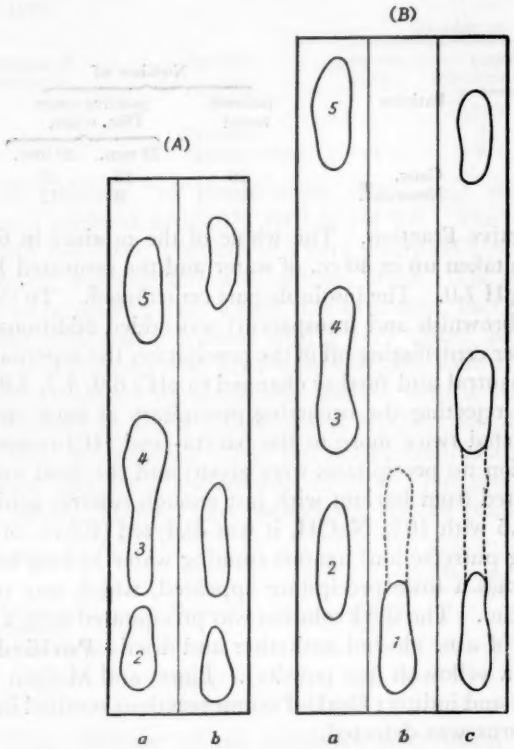

Fig. 1

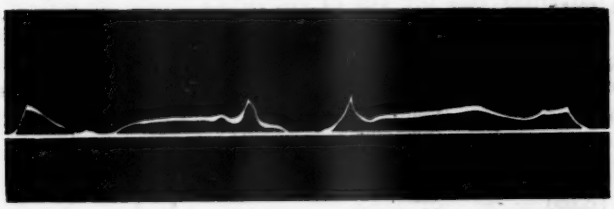

Fig. 2

Anode Cathode

Fig. 1. Chromatograms of sugar components of the Purified Active Fraction. (A) *n*-Butylacetate-pyridine-water (5:3:1) chromatograms; duration of test 8.5 hrs. (B) *n*-Butylacetate-acetic acid-water (3:2:1) chromatograms; duration of test 15.5 hrs. a in (A) and a and b in (B): standards. 1: chondrosamine sulfate, 2: glucosamine sulfate, 3: glucuronic acid, 4: galactose, 5: mannose.

Fig. 2. Electrophoretic pattern of a 1% solution of the Purified Active Fraction in phosphate buffer, pH 7.8, I=0.29; 20°C; current 19 ma. Exposure 1 hour and 20 minutes after starting current. Ascending limb to the left.

$$[\alpha]_D^8=\frac{-0.80\times 100}{(1.55/1.5)\times 1}=-77.4^\circ \text{ (in water)}$$

Paper partition chromatography of the sugar components

50 mg. of the substance in 5 cc. of 1 N H_2SO_4, as sealed in an ampoule, were heated in a boiling water-bath for 5 hours. The hydrolysate was nearly neutralized with the saturated $Ba(OH)_2$ and the filtrate (pH 5.8) distilled in vacuo to 1 cc. Further operation was effected kindly by Dr. Yosizawa according to his technique.[3] The volumes of the condensate applied on paper strips, 0.025 cc. Solvents for running, *n*-butylacetate-pyridine-water (5:3:1) and *n*-butylacetate-acetic acid-water (3:2:1). Solvent fronts run 40 cm. Developer, $AgNO_3$-ammonia. Temperature 19–20°C. The distribution of the sugar spots in the chromatograms are set out diagrammatically in Figs. 1A and 1B.

When run by those solvents, glucuronic and galacturonic acid are hardly distinguished from each other, only demonstrating a hexuronic acid, whereas glucosamine and chondrosamine are separated remote enough by the second solvent. The outcomes of the chromatography indicated the presence of chondrosamine, a hexuronic acid and mannose.

Electrophoretic analysis

Electrophoresis showed four boundaries (Fig. 2).

Summary

The watery extract of placentae was fractionated and the substance that gives the skin reaction of cancer and pregnancy was found involved in a protein fraction soluble in glacial acetic acid. Some chemical and physical properties of this fraction were examined.

The expenses of this work were defrayed in part by a grant given from the Education Department through the Grant Committee of the Science Council.

H. Masamune.

References and Notes

1) Matsubara, Nippon Iji Shimpo 1946, No. 1202, 5; 1949, No. 1301, 7; 1951, No. 1401, 14; Sogo Igaku 1947, **4**, 504; Nippon Shokakibyo Gakkai Zasshi, 1950, **47**, Nos. 3 & 4, 9; 1950, **47**, Nos. 9 & 10, 7; and else.

2) The violet coloration was stained dark so that accurate color-comparison was not allowed.

3) Yosizawa, Tohoku J. Exp. Med., 1950, **53**, 128.

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On the Placental Substance Giving the Skin Reaction of Cancer and Pregnancy

Second Paper*

By

Masayosi Ogawa
(小川 政良)

(From the Medico-chemical Institute (Director: Prof. H. Masamune) and the Surgical Clinic of Prof. M. Muto, Tohoku University, Sendai)

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To separate the substance in question in a homogeneous state, the writer made several trials. As a preliminary experiment the glacial acetic acid-soluble, phosphorus-free fraction (See First Paper[1]) was digested with trypsin and also treated with formaldehyde to see the effect of these operations on that biological potency, with results suggesting protein nature of the active factor. Exhaustion of the fraction with hot benzene, chloroform and alcohol did not diminish its activity. On the other hand, heating of the material as dissolved in physiological saline at 100°C, irradiation with x-ray and freezing with liquid air also did not inactivate it, namely, the active factor appeared fairly stable.

To isolate the principle, attempts were made to subdivide the fraction by electrodialysis, by precipitation with acid and with several protein precipitants, taking the electrophoretic pattern and outcome of the skin test as indications. Half saturation with $(NH_4)_2SO_4$, saturation with NaCl, addition of trichloroacetic acid up to 5% and electrodialysis all gave a precipitate that was heterogeneous, though more reactive to cancer patients than to non-cancerous. In efforts, however, a product was given, whose electrophoresis demonstrated only one component, by a combined procedure consisting in fractionated precipitation with Na_2SO_3 of the solution of the electrodialytic precipitate reprecipitated at pH 4.5. Its properties will be enumerated below, which afforded a proof that it belongs to the class of globulins.

Experimental

I. Material

A batch of the glacial acetic acid-soluble fraction of placentae which

* Fifth Report of Masamune and coworkers' "On Proteins and Amino Acids."

gave the cancer skin-reaction was separated and purified as described in the foregoing communication.[1]

II. Experiments concerning the Characters of the Active Factor

The technique of the skin test was the one employed already.[1]

1) Tryptic digestion. Main run. To 20 mg. of the material in 2 cc. of water were added 20 mg. of trypsin Kahlbaum and the pH adjusted to 9.0 with 1% Na_2CO_3. After addition of 2 drops of toluene the solution was placed in a thermostat at 37°C. The lowering pH was returned to the original every 12 hours, and when no more change had occurred (6×24 hours from the commencement), the solution was freed from toluene by aeration, neutralized (with 1% HCl), and heated in a boiling water-bath for 10 minutes.

Skin test:

Dilution of substance	Patients	Number of pateients tested	Number of positive cases (discr. width, 20 mm.)
1:2,000	Canc.	6	0
	Non-canc.	4	0

Control 1. A similar solution containing the material and trypsin but not alkalized was immediately heated in a boiling water-bath for 10 minutes. When cold it was alkalized and placed with toluene in the thermostat. After the lapse of the same time as above (The pH remained unaltered), the solution was neutralized and again heated.

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases (discr. width, 20 mm.)
1:2,000	Canc.	6	5
	Non-canc.	6	0

Control 2. A 1% trypsin solution was alkalized to pH 9.0, and after addition of toluene, incubated at 37°C (No change of pH occurred), and then neutralized and heated.

Skin test:

Dilution of trypsin	Patients	Number of patients tested	Number of positive cases (discr. width, 20 mm.)
1:2,000	Canc.	4	0
	Non-canc.	3	0

2) Treatment with formaldehyde. To 1 cc. of a 2% solution of the material was added 0.1 cc. of 40% formaldehyde. No precipitation

happened. The mixture, which showed a pH of 6.0, was neutralized with 0.01 *N* NaOH and stood at 20°C for 2 hours. Then it was precipitated with 50 cc. of abs. alcohol and one drop of 30% acetic acid, washed with abs. alcohol four times and dried (in vacuo over H_2SO_4).

Skin test:

Dilution of substance	Patients	Number of patients tested	positive cases Discr. width 20 mm.	positive cases Discr. width 25 mm.
1:2,000	Canc.	11	2	0
	Non-canc.	19	9	0

3) Exhaustion with benzene, chloroform and alcohol. 100 mg. of the material were refluxed with 10 cc. of dry benzene for 30 minutes. While hot, the solution was separated, and the residue was treated twice more in a similar manner and dried. It was further treated once with chloroform and once with abs. alcohol. The final residue amounted to 75 mg. On the other hand, the parts dissolved in the organic solvents were recovered by distillation of the solutions together.

Skin test:

Subdivision	Dilution of substance	Pateints	Number of patients tested	positive cases (discr. width, 25 mm.)
The portion undissolved	1:2,000	Canc.	10	9
		Non-canc.	10	1 (discr. width, 20 mm.)
The portion dissolved	1:200*	Canc.	10	0
		Non-canc.	10	0

* Emulsified in physiological saline with a very dilute NaOH.

4) Heating. 7.5 mg. of the material in 15 cc. of physiological saline (The solution was neutral) were heated (in an ampoule) for 30 minutes at 100°C. Neither turbidity nor precipitation occurred. The solution was cooled and diluted to 0.05% for the skin test.

Skin test:

Dilution of substance	Patients	Number of patients tested	positive cases (discr. width, 25 mm.)
1:2,000	Canc.	12	8
	Non-canc.	12	2

5) Irradiation of x-ray (The writer is indebted to Dr. H. Kurosawa in the Department of Radiology). 5 mg. portions of the material were sealed with 10 cc. of physiological saline in a hard-glass ampoule and x-ray irradiated (70 kv, 2 ma, no filter, 192 r per minute, distance 15 cm.). Values of r irradiated were 1,000, 3,000, 5,000, 10,000, 50,000 and 100,000.

Neither coloration nor precipitation happened.

Skin test:

r value	Dilution of substance	Patients	Number of patients tested	Number of positive cases (discr. width, 25 mm.)
1,000	1:2,000	Canc.	3	2
		Non-canc.	3	1
3,000	"	Canc.	3	1
		Non-canc.	3	1
5,000	"	Canc.	3	3
		Non-canc.	3	0
10,000	"	Canc.	4	4
		Non-canc.	4	0
50,000	"	Canc.	4	2
		Non-canc.	4	1
100,000	"	Canc.	4	4
		Non-canc.	4	1

6) Freezing. 5 mg. of the material were taken up in 10 cc. of physiological saline. The solution was frozen by dipping the container in liquid air and stood for 15 minutes, followed by allowing to thaw in air. Freezing and thawing was repeated thrice more.

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases (disc. width, 25 mm.)
1:2,000	Canc.	10	8
	Non-canc.	10	1

III. Preparatory Trials for the Separation of the Active Factor

1) Electrodialysis

1.1 g. of the material were taken up in 120 cc. of water and electrodialyzed (200 volts) in a Pauli apparatus against changes of water until the pH change in the outer fluids stopped (24~36 hours were requisite). The occurring precipitate was centrifuged, washed twice with water (electrodialyzed as well) and with alcohol and dried—**Ppt_{ed}** (165 mg.; a non-sticky, hygroscopic, brownish powder). The supernatant was distilled in vacuo to a syrup and after dehydrating with alcohol, dried—**Sol_{ed}** (500 mg.). The fluids siphoned out of the anode- and cathode-compartment were combined separately according to the chamber, neutralized with NaOH and HCl respectively and after distillation, dried —**Pt_{an}** and **Pt_{cat}**.

Skin test:

Subdivision	Dilution of substance	Patients	Number of patients tested	Number of positive cases (discr. width, 22 mm.)
Ppt_{ed}	1:2,000	Canc.	12	10
		Non-canc.	11	0
Sol_{ed}	„	Canc.	21	1
		Non-canc.	16	0
Pt_{an}	„	Canc.	4	0
		Non-canc.	3	0
Pt_{cat}	„	Canc.	3	0
		Non-canc.	3	0

Chemical and physical properties of Ppt_{ed}

a) Chemical analyses. Biuret strongly positive, Molisch slightly positive, the direct Osaki-Turumi test as well as the indirect faintly positive. N 13.3%, hexosamine 3.9%, non-amino sugars as mannose 3.5% (indole method), ash 2.0%.

b) Solubilities and precipitabilities. It did not dissolve completely in plain water. The clear centrifuged-supernatant of the turbid solution gave a precipitate on acidification with acetic acid (maximum precipitation at pH 4.5). To examine the precipitabilities a clear 1% solution (pH 8.0) prepared by the aid of NaOH was used. It was precipitated by $(NH_4)_2SO_4$ (at half saturation), lead acetate and tannic and picric acid and also by such organic solvents as ethanol, methanol and a mixture of equal volumes of acetone and ether but not by acetone alone. No precipitation happened when the solution was boiled after addition of a little NaCl, but on following acidulation a voluminous precipitate appeared that did not dissolve again by alkalization.

c) Electrophoretic analysis (See Fig. 1). It was proved non-homogeneous.

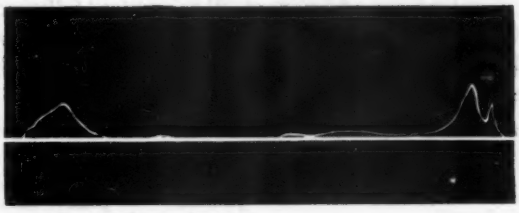

Fig. 1. An electrophoretic pattern of a 1% solution of Ppt_{ed} in phosphate buffer, pH 7.8, I=0.29; 20°C; 21 ma. Exposure 40 minutes after starting current. Ascending limb to the left.

Composition of Sol_{ed}

N 13.3%, hexosamine 7.1%, non-amino sugars as mannose (orcinol method) 7.9%, ash 0.4%.

2) Fractionation with $(NH_4)_2SO_4$

0.3 g. of the material was dissolved in 10 cc. of water and 10 cc. of the saturated $(NH_4)_2SO_4$ added. After 30 minutes the precipitate was centrifuged and washed with two 10 cc. portions of the half-saturated $(NH_4)_2SO_4$. It was then taken up in 20 cc. of water and dialyzed against running water until NH_4-(Nessler) and SO_4-ion were no more detected, followed by distillation in vacuo. The residue was washed with alcohol and dried—**Ppt$_{(NH_4)_2SO_4}$**, 67 mg. The portion remaining in the mother fluid was recovered by dialysis and distillation—**Sol$_{(NH_4)_2SO_4}$**, 130 mg.

Skin test:

Subdivision	Dilution of substance	Patients	Number of patients tested	Number of positive cases (discr. width, 23 mm.)
Ppt$_{(NH_4)_2SO_4}$	1:2,000	Canc.	21	20
		Non-canc.	11	4
				(discr. width, 25 mm.)
Sol$_{(NH_4)_2SO_4}$	„	Canc.	17	0
		Non-canc.	21	1

Composition

Ppt$_{(NH_4)_2SO_4}$: N 11.1%, hexosamine 3.7%, non-amino sugars as mannose (orcinol method) 5.3%, ash 5.4%.

Sol$_{(NH_4)_2SO_4}$: N 10.0%, hexosamine 7.8%, non-amino sugars as mannose (orcinol method) 10.8%, ash 9.6%.

Since the active principle was found precipitable by half saturation of $(NH_4)_2SO_4$, reprecipitations were carried out:—1.5 g. of the material were dissolved in 10 cc. of water and after precipitation and washing as above, again dissolved by addition of 10 cc. water, followed by precipitation with 10 cc. of the saturated $(NH_4)_2SO_4$ and washing with the half-saturated $(NH_4)_2SO_4$. After another reprecipitation 160 mg. of a non-sticky, brownish powder were given by dialysis and so on.

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases (discr. width, 22 mm.)
1:2,000	Canc.	32	25
	Non-canc.	44	10

Composition

N 12.4%, hexosamine 1.6%, non-amino sugars as mannose (orcinol method) 2.6%, ash 9.4%.

Electrophoresis (See Fig. 2). The product twice reprecipitated was found still extremely heterogeneous.

Remark: Even 30 reprecipitations were processed, but the product, which caused a redness with a larger width in most cancerous patients than in non-cancerous, gave the positive Molisch reaction, though faint, besides the positive biuret.

3) Fractionation with trichloroacetic acid

200 mg. of the material were agitated in 8 cc. of 5% trichloroacetic acid. The centrifugate was washed with two 5 cc. portions of 5% trichloroacetic acid. It was followingly taken up in 5 cc. of water,

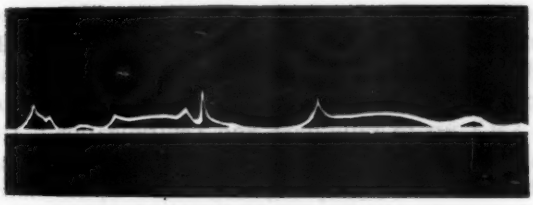

Fig. 2. An electrophoretic pattern of the product twice reprecipitated by half saturation of $(NH_4)_2SO_4$. 1% solution in phosphate buffer, pH 7.8, I=0.29. 13°C. 17.5 ma. Exposure 45 minutes after starting current. Ascending limb to the left.

acidified with 2% HCl to pH 2.0, removed from the insoluble and precipitated with 50 cc. of abs. alcohol with subsequent washing with alcohol —**Ins_{CCl_3COOH}**. Yield 40 mg. The part that dissolved in the trichloroacetic acid solution was recovered by neutralization of the solution, dialysis, vacuum distillation to dryness and washing with alcohol—**Sol_{CCl_3COOH}**. Yield 85 mg.

Skin test:

Subdivision	Dilution of substance	Patients	Number of patients tested	Number of positive cases (discr. width, 24 mm.)
Ins_{CCl_3COOH}	1:2,000	Canc.	8	6
		Non-canc.	5	0
				(discr. width, 22 mm.)
Sol_{CCl_3COOH}	"	Canc.	7	6
		Non-canc.	4	0

Composition

Ins_{CCl_3COOH}: N 12.3%, hexosamine 3.6%, non-amino sugars as mannose (orcinol method) 6.7%, ash 12.3%.

Sol_{CCl_3COOH}: N 11.9%, hexosamine 4.6%, non-amino sugars as mannose (orcinol method) 11.0%, ash 5.9%.

Thus it appeared that this precipitant is of no use.

4) Fractionation of a Ppt_{ed} (the part precipitated by electrodialysis (See 1)) with NaCl after precipitation with acid

1.2 g. of a Ppt_{ed} like the one in 1) were taken up in 20 cc. of water by means of NaOH (added to a pH of 9.0) and after rejecting a trace of the insoluble part, the clear solution was acidified to pH 4.5 (I.E.P.) with 2% HCl, whereby a deposit appeared. The centrifugate was washed with a very dilute HCl of pH 4.5 twice and with alcohol and dried. It was precipitated once more in a similar manner, then dissolved perfectly at pH 6.0 by addition of NaOH and electrodialyzed again. The precipitate was washed with electrodialyzed water and alcohol as before and dried. 200 mg. was the yield at this stage. The present product was dissolved in 20 cc. of water by using a dilute NaOH (The solution was of pH 8.0) and saturated with NaCl, then the precipitate here was further subjected four times to suspension in 10 cc. of water, following addition of NaCl up to saturation and centrifugation. It was finally washed with a 40 cc. portion of water and many changes of methanol.

The substance contained pretty much of ash, so that it was electrodialyzed over again—$\mathbf{Ppt_{NaCl}}$. 65 mg. of substance were given.

Electrophoresis (Fig. 3) showed three components.

5) Fractionation of Ppt_{ed} with Na_2SO_3 after prior precipitation with $(NH_4)_2SO_4$ (at half saturation)

100 mg. of the Ppt_{ed} (See 4)) were dissolved in 5 cc. water (20°C) by the aid of NaOH and 5 cc. of the saturated $(NH_4)_2SO_4$ (20°C) added. Stood at 20°C for 30 minutes. The precipitate was reprecipitated as described in 2) five times and then after dissolution in 7 cc. of water by

Fig. 3

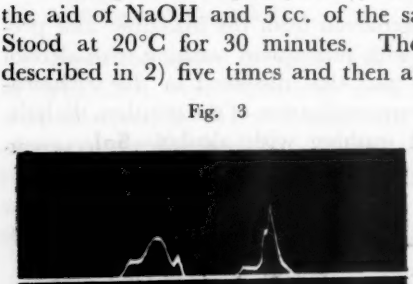

Fig. 4

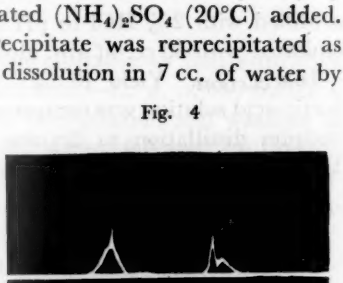

Fig. 3. An electrophoretic pattern of Ppt_{NaCl} in phosphate buffer, pH 7.8, I=0.29; 12°C; 17.5 ma. Exposure 40 minutes after starting current. Ascending limb to the left.

Fig. 4. An electrophoretic pattern of a 1% solution of $Ppt_{17\cdot5}$ in the same buffer as above; 18°C; 17.5 ma. Exposure 40 minutes after starting current. Ascending limb to the left.

faint alkalinization (NaOH was used; pH of the solution 7.8), removed from some insoluble and electrodialyzed. The precipitate here was washed and dried as already noted—60 mg. The substance thus obtained was next taken up in 3 cc. of water (20°C) and 28% Na_2SO_3 (20°C) added to 17.5%. Stood for 30 minutes at 20°C and centrifuged. The centrifugate was washed with 17.5% Na_2SO_3 at the same temperature twice, dissolved in 7 cc. of water as above and electrodialyzed over again. The product amounted to 42 mg.—**$Ppt_{17.5}$**. It proved electrophoretically to comprise two components (Fig. 4).

6) Fractionated precipitation of an electrodialytic precipitate with Na_2SO_3

i) 380 mg. of a Ppt_{ed} were taken up in 20 cc. of water as above, and the transparent brownish solution freed from a trace of an insoluble part was precipitated by cautious addition of 2% HCl to pH 4.5 and washed with a dilute HCl of the same pH three times. Electrodialysis of it as dissolved in 150 cc. of water gave a precipitate weighing 200 mg. This was suspended in 8 cc. of water (20°C) and 0.5 cc. of 28% Na_2SO_3 (20°C) added in drops whereby a clear solution formed. The Na_2SO_3 solution was added furtheron. When the concentration of the salt reached 13.26% (The total volume of the salt solution used was 7.2 cc.), addition of the salt was stopped. Stood for 30 minutes at 20°C. The precipitate was centrifuged (The mother fluid—**$Fl_{13.26}$**—was set aside) and washed twice with 13.26% Na_2SO_3 (20°C), then it was dissolved in 20 cc. of water (dissolved transparently) and dialyzed (for 24 hours) at first and afterwards electrodialyzed. The precipitate (in the inner compartment) was separated, washed and dried—60 mg., **$Ppt_{13.26}$**. ii) The mother fluid $Fl_{13.26}$ was precipitated by increasing the Na_2SO_3 concentration to 15.36% (The mother fluid here will be referred to as **$Fl_{15.36}$**) followed by a series of operations like above—**$Ppt_{15.36}$**, 25 mg. iii) In a similar manner, 22 mg. of a precipitate at 16.78% Na_2SO_3 were given from $Fl_{15.36}$—**$Ppt_{16.78}$**.

Electrophoresis. The patterns (Fig. 5) showed that $Ppt_{13.26}$ was practically homogeneous contrary to the other Ppts.

As will be explained, $Ppt_{13.26}$ was active regarding the biological reaction.

IV. Isolation of the Active Principle and Its Properties

Preparation

A batch of $Ppt_{13.26}$'s (yellowish-brownish powders) was prepared as described in III, 6). The average yield was 175 mg. per g. of Ppt_{ed} (See III, 1)).

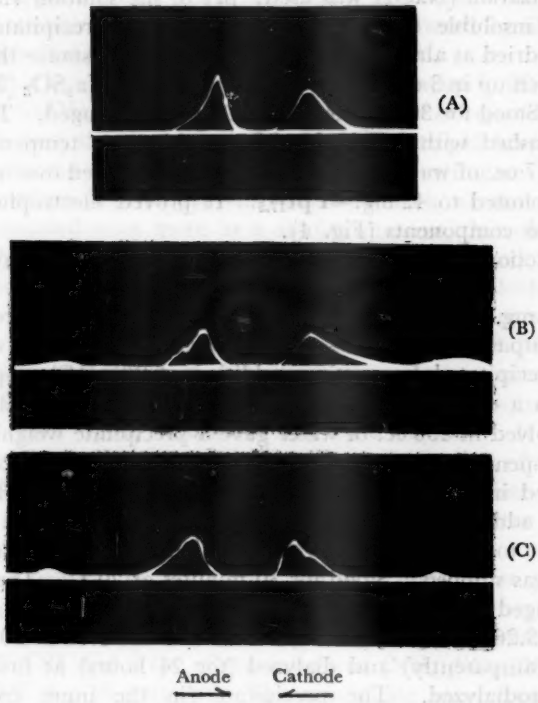

Fig. 5. Electrophoretic patterns of the products after fractionated precipitation with Na_2SO_3; 1% solutions in phosphate buffer, pH 7.8, I=0.29. (A) $Ppt_{13.26}$: 18°C, current 12 ma, exposure 50 minutes after starting current, mobility 12.5 $cm^2 \cdot volt^{-1} \cdot sec^{-1} \cdot 10^5$. (B) $Ppt_{15.36}$: 13°C, current 17.5 ma, exposure 40 minutes after starting current. (C) $Ppt_{16.78}$: 13°C, 17.5 ma, exposure 40 minutes after starting current. Ascending limbs to the left.

Properties

Skin test:

Dilution of substance	Patients	Number of patients tested	Number of positive cases (discr. width, 23 mm.)
1:2,000	Canc.	10	9
	Non-canc.	10	2

Chemical qualitative reactions

Biuret strongly positive; xanthoprotein, Sakaguchi, Hopkins-Cole, Millon, reduced sulfur and Pauly diazo also positive. The positive Molisch test was given, but neither Goldschmiedt (for hexuronic acid) nor Neuberg-

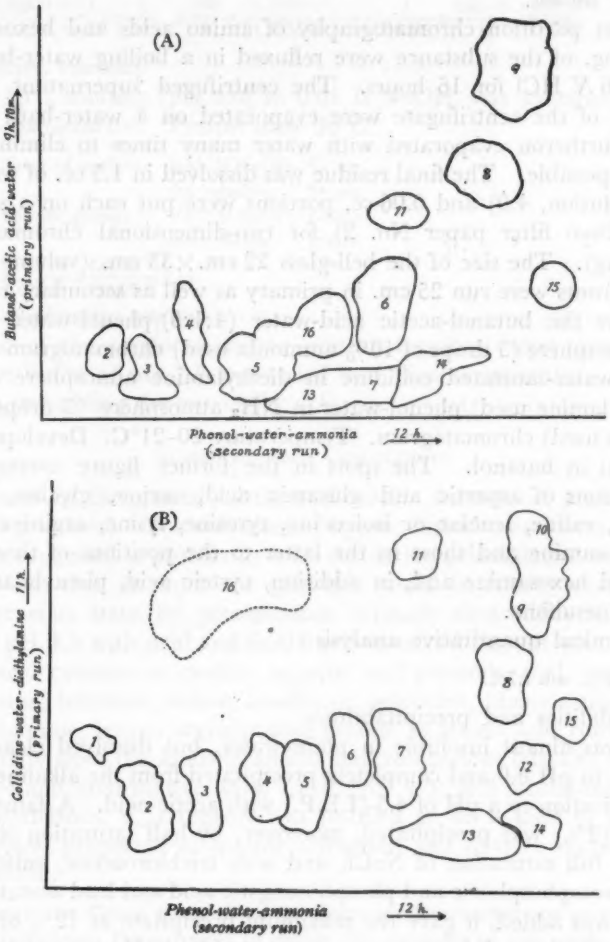

Fig. 6. Chromatograms of the hydrolysate of the active principle. (A) Butanol-acetic acid-water/phenol-water-ammonia chromatogram. (B) Collidine-water-diethylamine/phenol-water-ammonia chromatogram. Developer ninhydrin. 1: cysteic acid, 2: aspartic acid, 3: glutamic acid, 4: serine, 5: glycine, 6: alanine, 7: histidine, 8: valine, 9: leucine or isoleucine, 10: phenylalanine, 11: tyrosine, 12: methioninesulfone, 13: lysine, 14: arginine, 15: proline, 16: hexosamine.

Saneyoshi (for hexuronic acid) nor aniline acetate (for pentose and hexuronic acid) nor Osaki-Turumi (the direct as well as the indirect) reac-

tion was shown.

Paper partition chromatography of amino acids and hexosamine

25 mg. of the substance were refluxed in a boiling water-bath with 3 cc. of 6 *N* HCl for 16 hours. The centrifuged supernatant and the washings of the centrifugate were evaporated on a water-bath and the residue furtheron evaporated with water many times to eliminate HCl as far as possible. The final residue was dissolved in 1.5 cc. of water (pH of the solution, 4.0) and 0.06 cc. portions were put each on a square of paper (Toyo filter paper No. 2) for two-dimensional chromatography (ascending). The size of the bell-glass 22 cm. × 33 cm. (volume 13.5 *l*.). Solvent fronts were run 25 cm. in primary as well as secondary run. Fig 6A shows the butanol-acetic acid-water (4:1:5)/phenol-water (4:1) in NH_3 atmosphere (3 drops of 10% ammonia used) chromatogram and Fig. 6B the water-saturated collidine in diethylamine atmosphere (3 drops of diethylamine used)/phenol-water in NH_3 atmosphere (3 drops of 10% ammonia used) chromatogram. Temperature 20–21°C. Developer, 0.2% ninhydrin in butanol. The spots in the former figure correspond to the positions of aspartic and glutamic acid, serine, glycine, alanine, histidine, valine, leucine or isoleucine, tyrosine, lysine, arginine, proline and hexosamine and those in the latter to the positions of those amino acids and hexosamine and, in addition, cysteic acid, phenylalanine and methioninesulfone.

Chemical quantitative analysis

N 14.3%, ash 1.7%.

Solubilities and precipitabilities

It was almost insoluble in plain water, but dissolved clearly when alkalized to pH 8.0 and completely precipitated from the alkaline solution on acidification to a pH of 4.5 (I.E.P.) with acetic acid. A faint alkaline solution (2%) was precipitated, moreover, by half saturation of $(NH_4)_2SO_4$ and full saturation of NaCl, and with trichloroacetic, sulfosalicylic, tannic, metaphosphoric and phosphotungstic acid and lead acetate. When Na_2SO_3 was added, it gave the maximum precipitate at 12% of the salt. Picric acid alone did not cause even turbidity. Heat coagulation did not take place at the own pH of the solution (See also 1),b) in the former section). The substance was insoluble in organic solvents (alcohol, ether, acetone).

Specific refraction

15.0 mg. of the substance were dissolved in 1.5 cc. of 0.01 *N* NaOH. The pH of the solution was 7.8. This 1% solution was examined by means of an Abbe refractometer at 17.5°C.

n 1.33551, n_o 1.33366, d 1.0034, d_o 1.0005, p 1.0.

$$\frac{(1.33551)^2-1}{(1.33551)^2+2}\times\frac{1}{1.0034}\times\frac{100}{1}-\frac{(1.33366)^2-1}{(1.33366)^2+2}\times\frac{1}{1.0005}\times\frac{100-1}{1}=0.253°.$$

Relative viscosity

A 0.25% solution (pH 9.0) in 0.01 *N* NaOH was examined by an Ostwald viscosimeter. Temperature 20°C.

Time of outflow: 0.01 *N* NaOH, 47 sec.
the solution, 54 sec.
Specific gravity: 0.01 *N* NaOH, 1.0005
the solution, 1.0018

$$\eta_{rel}=\frac{54}{47}\times\frac{1.0018}{1.0005}=1.15.$$

Electrophoretic analysis

Some of the products (before putting together) were dissolved in phosphate buffer of pH 7.8 and tested in a Tiselius apparatus. All gave a pattern resembling the one in Fig. 5A.

Summary and Conclusion

A batch of the glacial acetic acid-soluble fraction of placental extracts was prepared. The outcomes of tryptic digestion and formaldehyde treatment suggested a protein in it as the active principle for the cancer reaction and after attempts the principle could be finally separated in a homogeneous state by precipitation through electrodialysis, precipitation at pH 4.5 with acid and final fractionated salting-out with Na_2SO_3. It contained cysteine or cystine, aspartic and glutamic acid, serine, glycine, alanine, histidine, valine, leucine or isoleucine, phenylalanine, tyrosine, methionine, lysine, arginine and proline as amino acid components.

Not only from the kinds of amino acid component but also from the qualitative and quantitative data, I.E.P. (pH 4.5) and solubilities as well as precipitabilities, it is regarded as justified to be a kind of globulin. Such physical properties as specific refraction and relative viscosity were also measured.

Through the Grant Committee for Scientific Researches was given a grant from the Education Department to defray the expenses of this work, which is gratefully acknowledged. H. Masamune.

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The Tohoku Journal of Experimental Medicine, Vol. 56, Nos. 1-2, 1952

Studies on the Respiratory Arrhythmia of Man
Its Relation to Respiratory Depth, Cardiac Rate and to the Ages of Individuals

By

Kojiro Matsuda and **Taizo Suzuki**
(松田 幸次郎) (鈴木 泰三)

(From the Laboratory of Applied Physiology, Faculty of Medicine, Tohoku University, Sendai; Director: Prof. K. Matsuda)

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Phasic variation of the instantaneous cardiac rate, synchronous with respiratory movement was first described by Ludwing as early as the middle of the last centrury, and is now generally known as "Respiratory arrhythmia." This type of arrhythmia is one of the sinus arrhythmias and can be observed more or less in every normal individual, so far as the cardiac rhythm is carefully examined. The cardiac action usually quickens in the inspiratory phase and slows down in the expiratory phase of respiration.

Many studies have been performed pertaining this type of arrhythmia, especially as to its physiological mechanism. E. Hering[6] and others attributed it to the reflexes originating in the lungs. On the contrary, other authors such as Fredericq,[4] Foa[3] and C. Heymans,[6] insisted on the theory of central origin based upon the apparently undeniable fact that the arrhythmia appeared independently of the inflation movement of lungs. Recently also Vanremootre[16] regarded such central mechanim as the chief causative factor of the human respiratory arrhythmia.

Anrep, Pascual and Rössler,[1] however, confirmed by elaborate experiments in dogs that both the peripheral and central mechanisms were at work.

While many such studies thus far reported have dealt with the physiological mechanism of the respiratory arrhythmia, chiefly in animals under various experimental conditions, the systematic surveys on the influences, such as the depth of respiration, cardiac rate etc. upon the human respiratory arrhythmia have been performed but rarely or insufficiently. Such a circumstance is probably due to the fact that the instantaneous cardiac rhythm in the intact organism is so easily modified by diverse

and yet uncontrollable factors, intrinsic as well as extrinsic, that the data for quantitative evaluation of the respiratory arrhythmia by means of, for example, electrocardiographic or sphygmographic record often become of doubtful value. To obviate such inconvenience it is necessary to take a long series of record of instantaneous cardiac rhythm.

Fleisch's "Pulszeitschreiber"[9] which Vanremootre recently utilized, is an adequate instrument, but not perfect as the subject to be examined should keep his arm inmobile throughout the test. Such a procedure may often cause some strain in the subject physically as well as mentally, especially in case of observation extending to fairly long periods of time, say ten to twenty minutes.

"*Cardiotachograph*",[10] which was devised by one of the present authors, is free from such limitation and permits to record automatically all the instantaneous cardiac rates of man or animal, not only in rest but also in activities without forcing any stress, and moreover for any length of time.

In this communication are reported the results of the observations of the respiratory arrhythmia of man by means of Matsuda's *Cardiotachograph*, especially of the influences upon the arrhythmia of the depth of respiration and the cardiac rate, with some remarks on the influences of the age of individual.

Experimental

Methods

The subjects examined were all healthy Japanese, twenty five in number (comprising of twenty males and five females), covering the ages from seven to fifty-six.

The explanation of the instrument, "*Cardiotachograph*," is found elsewhere.[10] Fig. 1 shows one of its records. Each pulse interval (time interval between two consecutive ventricular systoles) is represented by the length of each vertical line. The numerical values of the pulse intervals in unit of second can be read by the scale at the left.

Respiratory movement was always recorded simultaneously by means of a stethograph.[8] In cases where the accurate volume of tidal air was necessary, it was registered by recording the movement of a calibrated spirometer. Fig. 2 shows such a record.

All subjects were examined in recumbent, sitting and standing postures, all under the condition of complete rest and ease. In the recumbent and sitting postures, the records were taken continuously for over ten minutes after the preliminary ten to twenty minutes' rest. The examination in the standing posture was carried out for at least five minutes

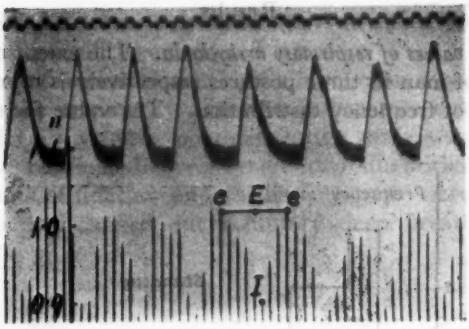

Fig. 1. Record of instantaneous cardiac rate (Cardiotachogram) of a healthy man in recumbent posture.
Upper: time in two seconds. Middle: respiratory record, the upward movement is inspiration. Lower: cardiotachogram. Numerical value for each pulse interval can be read by the scale at the left. E: expiratory pulse interval (average of two *s*s.). I: inspiratory pulse interval. The difference between E and I is the measure of respiratory arrhythmia.

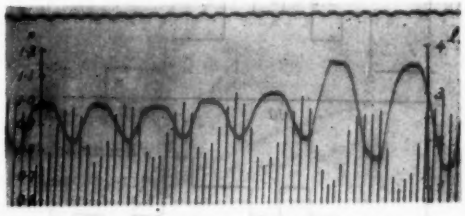

Fig. 2. Simulatneous recording of respiratory tidal volume and cardiotachogram. Upper: time signal, every two seconds. Middle: quantitative record of respiratory depth. Its scale is shown at the right. Lower: cardiotachogram, the scale for which is at the left.

beginning exactly three minutes after the subject stood up from the sitting position. The changes in cardiac rhythm in the transitional phase of posture change are not considered in this report.

Each record obtained was first inspected as a whole, and the parts thereof indicating apparently irregular and gross changes of respiration or of cardiac rhythm were excluded from study.

The extent of the respiratory arrhythmia was expressed quantitatively by the maximum difference between the expiratory and the inspiratory pulse intervals. Here the expiratory pulse interval means the average of two values for the expiratory phases preceding and following the inspiration (See Fig. 1).

Results

1. *Normal values of respiratory arrhythmia.* The extent of the respiratory arrhythmia of man in three postures respectively is represented in Fig. 3 as a diagram of frequency distribution. The value for each individual

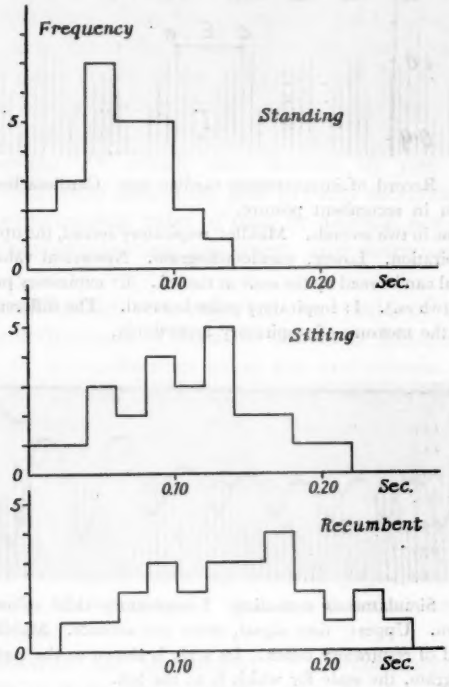

Fig. 3. Respiratory arrhythmia of man in rest and in three postures. Abscissa: respiratory arrhythmia measured by the difference of pulse intervals in expiratory and inspiratory phases. Ordinate: frequency of occurrence among 25 individuals.

is the average of data for more than twenty consecutive respiratory cycles. There was no case where the respiratory arrhythmia was not observed at all, though the arrhythmia in a few subjects was so slight as to amount only to 0.02 seconds.

The arrhythmia generally varies in extent according to the postures. It decreases in the order from the recumbent to the sitting, then to the standing postures. The maximum respiratory arrhythmia observed was 0.258 sec. in a lying person.

No essential difference was found between males and females. The influence of the age will be considered later.

2. *Influence of respiratory depth.* While it seems to be a natural consequence that the respiratory arrhythmia becomes exaggerated by an increase of tidal air volume, no quantitative study was reported so far concerning the relations between these two quantities. In ten adult men the volumes of tidal air and cardiotachogram were simultaneously recorded when they carried out voluntary deep breathings of various depth (Fig. 3).

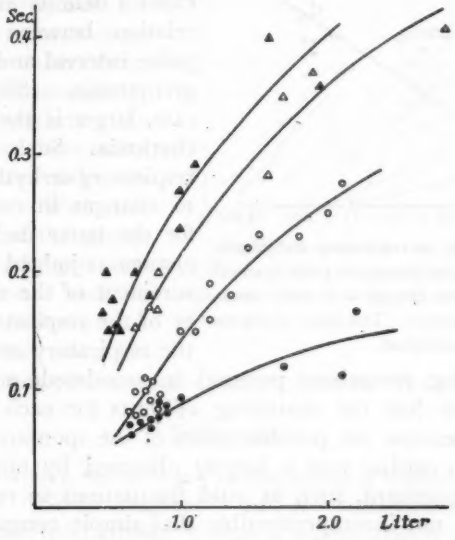

Fig. 4. Effect of respiratory depth (abscissa) on the respiratory arrhythmia (ordinate). Here are shown examples from four subjects as distinguished by four different symbols.

The representative results thereof are shown in Fig. 4. While the respiratory arrhythmia gets marked with hyperpnea, the interrelationship is not linear. The correlation curves run usually paraboloid courses which are different according to the individuals. Generally speaking, the more marked the respiratory arrhythmia for the same respiratory tidal volume, the more marked is the slope of the curve.

3. *Influence of the cardiac rate.* It has long been stated that the respiratory arrhythmia gets conspicuous in case of bradycardia (Lewis[9] and Friedberg[8] and others). Shima[10], however, by studying the pulse

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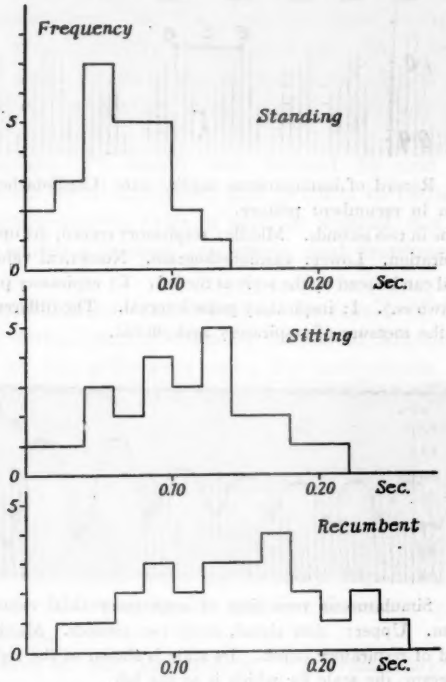

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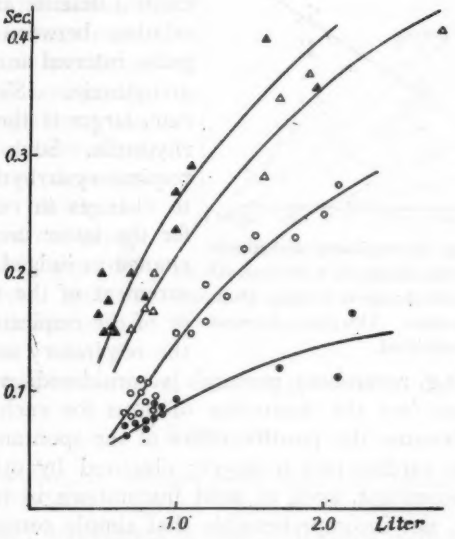

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3. *Influence of the cardiac rate.* It has long been stated that the respiratory arrhythmia gets conspicuous in case of bradycardia (Lewis[9] and Friedberg[5] and others). Shima[18], however, by studying the pulse

rates in great numbers of young men, obtained a different result that there was no definite correlation between the pulse rate and the respiratory arrhythmia.

The present authors reexamined this point by measuring the changes in respiratory arrhythmia accompanying the changes in cardiac rate in one and the same individual. To modify the cardiac rate, the posture of the individual was changed from recumbent to sitting, then to standing, as the cardiac rate usually increased in the order mentioned.

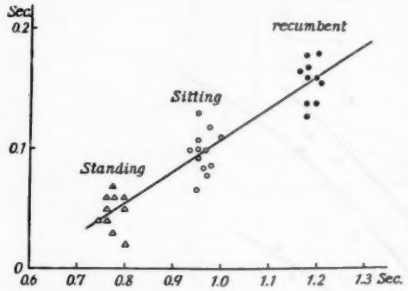

Fig. 5. Change in respiratory arrhythmia (ordinate) due to the change in pulse interval (abscissa). The latter change is brought about by the change in posture. This figure shows an example from one individual.

Fig. 5 is a typical example. As seen clearly in this figure, there exists a definite and almost linear relation between the expiratory pulse interval and the respiratory arrhythmia. Slower the heart rate, larger is the respiratory arrhythmia. Such changes in the respiratory arrhythmia are not due to changes in respiratory depth, for the latter undergoes no gross change as judged by actual measurement of the tidal air volume or of the respiratory rate. When the respiratory arrhythmia in one posture only (e.g. recumbent posture) is considered, no such clear-cut relation appears (see the scattering of dotts for each posture in Fig. 5.), probably because the possible effect of the spontaneously occurring small change in cardiac rate is largely obscured by other trifle factors which are concomitant, such as mild fluctuations in respiratory depth. It is, therefore, not incomprehensible that simple comparison of values of various individuals obtained by measuring the arrhythmia during a few respiratory cycles in one posture only, as Shima performed, does not yield any definite information.

Let the correlation curve between the cardiac rate (or pulse interval) and the respiratory arrhyrhmia as shown in Fig. 5 be called *Respiratory Arrhythmia Characteristic* (RAC). The RAC is linear, approximately but not strictly. In some subjects whose cardiac rates are extremely slow (e.g. 40 or less per minute) especially in the recumbent posture, the respiratory arrhythmia does not proportionately increase with bradycardia so that the RAC curve becomes gradually flat, showing that there is a certain limit in extent of the respiratory arrhythmia. A similar fact was observed by Anrep and others in their experiments on dogs.[1)]

The slope of the RAC in its range of approximate linearity is different

from one individual to another, especially according to their ages. This point will be considered in the next section.

4. *Influence of age of individual upon the respiratory arryhthmia.* Fig. 6 shows RAC of five different groups of persons classified according to their ages; group A: 7 to 14 yrs, group B: 15 to 30 yrs, group C: 31 to 40 yrs, group D: 41 to 50 yrs and group E: above 51 yrs. These RAC curves were obtained by measuring the respiratory arrhythmia in three postures as above mentioned, and the values from several persons in each group were plotted together. As seen in this figure, there exists a certain influences of the age upon the RAC. With the advance in age of the group the slope of RAC gets decreased. However, such is only a general tendency and not the absolute rule, for there were at times encountered cases exceptional to this rule.

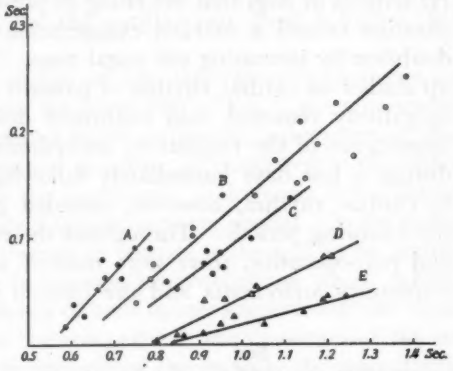

Fig. 6. Respiratory Arrhythmia Characteristic (RAC) for different age groups (see text). Abscissa: expiratory pulse interval. Ordinate: respiratory arrhythmia.

Discussion

As reported above, the authors' observations of human cardiac rhythm by means of the "*Cardiotachograph*" lead to confirmation of the opinion that the respiratory arrhythmia of man is quantitatively related not only to the depth of respiration but also to the cardiac rate. Without doubt the slowing of heart action brings about a tendency to increase, and the tachycardia a tendency to decrease the respiratory arrhythmia. This is in accord with the animal experiments by Anrep and others[13] and by Sato[11]. The latter author in this laboratory examined the respiratory arrhythmia of dogs in experimental hyper- and hypo-thyroidism which were inducd by feeding thyroid glands and by thyroidectomy respectively. Taguchi, who studied the patterns of cardiac rhythm of patients of hy perthyroidism in courses of therapy (iodide or thiouracil administration as well as subtotal thyroidectomy), also confirmed a distinct relationship between the respiratory arrhythmia and cardiac rate.[14] According to the concept prevailing at present, the respiratory arrhythmia is brought about chiefly, if not entirely, by the inhibition of the cardiac vagus center

in the medulla by the reflex from the lungs and/or by direct effect of the respiratory center. Whatever the primary mechanism of the vagus inhibition may be, the above mentioned relation between the respiratory arrhythmia and the pulse rate will not be difficult to expect.

In connection to this, Schweitzer's observation[12] is worthy to note. He noticed in dogs that the rising in pressure inside the carotid sinus preparation caused a marked exaggeration of the repiratory arrhythmia, doubtless by increasing the vagal tone. Tanemura[15] carried out follow-up studies on cardiac rhythm of patients whose carotid sinus nerves were operatively removed, and confirmed definite decrease or complete disappearance of the respiratory arrhythmia with concomitant tachycardia during a few days immediately following the operation. Such change in cardiac rhythm, however, subsided gradually and spontaneously in the following periods. Throughout the entire course comprising the pre- and post-operative, there were noticed a distinct relation between the respiratory arrhythmia and the cardiac rate.

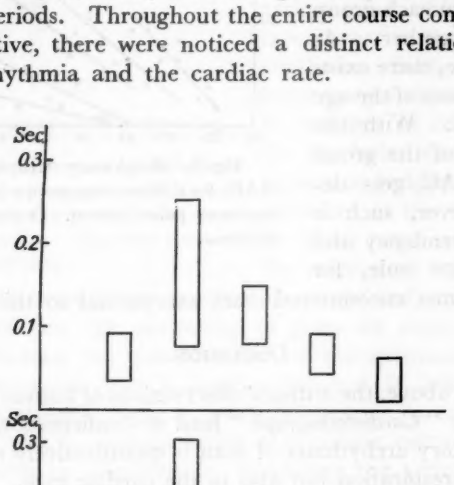

Fig. 7. Respiratory arrhythmia for different age groups (upper figure) compared with change in heart rate (expressed by pulse interval) brought about by change in posture, from recumbent to sitting (lower figure).

Shima's opinion against such correlation is clearly erroneous, perhaps due to inadequacy in the method and in the treatment of materials for

observation.

The respiratory arrhythmia has been known to be particularly marked in younger individuals as it was called by Sir MacKenzie " Youthful type of arrhythmia." In fact, as shown in Fig. 7, the respiratory arrhythmia is most marked in the group B (adolescent age). Though the slope of RAC for children is as marked as for adolescent subjects, the respiratory arrhythmia in children is less, for the cardiac rate itself is higher (See Fig. 6).

Concerning the influence of age on the respiratory arrhythmia an interesting fact was observed. In the lower half of Fig. 7 are represented the changes in heart rate (experessed by its reciprocal, pulse interval) due to change of posture from recumbent to sitting, as compared to the upper half of the figure which shows the respiratory arrhythmia for different age groups. The patterns of both diagrams are very similar. As the initial change in cardiac rate on the change in posture is thought to be mainly evoked by reflexes originating in various pressoreceptors which modify the cardiac vagal tone, the similarity of both figures suggests that the vagal tone plays the dominant, if not the sole, rôle in the causation of the respiratory arrhythmia in man. Superiority of the vagus to the sympathetic nerve in the genesis of the respiratory arrhythmia was stressed by Anrep and others[1] based on their experiments on dogs.

Summary

1. By means of " *Cardiotachograph* " the quantitative study on the respiratory arrhythmia of healthy men (covering ages from seven to fifty-six) was performed. The observations were carried out in rest and in three postures, viz., recumbent, sitting and standing.

2. The respiratory arrhythmia increases with the respiratory depth. The relations between these two can be represented generally as paraboloid curves, slopes of which however, being different according to the individuals.

3. The respiratory arrhythmia is definitely influenced also by the cardiac rate: the bradycardia usually enforces the arrhythmia. The relationship between the respiratory arrhythmia and the pulse interval is nearly linear, except in state of extreme slowing of the heart. The slope of the correlation line is more marked for younger than for older persons.

4. Among all age groups of subjects, the respiratory arrhythmia is most marked as well as most variable in adolescence. The same patterns for various age groups can be observed in the changes in cardiac rate due to the change in posture.

All these results were discussed briefly in connection to the physiological mechanism of the respiratory arrhythmia.

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